Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 10, No. 1, 232-257
2026
Publisher: Learning Gate
DOI: 10.55214/2576-8484.v10i1.11607

Impact of isoflavones and probiotics on human gut health and bone health: A review

Thivya, J[1], Deepa, S[1*], Adeyeye, Samuel Ayofemi Olalekan[1]
[1]Department of Food Technology, Hindustan Institute of Technology and Science; deepa.dee1920@gmail.com (D.S..) saadeyeye2015@gmail.com (A.S.A.O.).

Abstract: This review aims to evaluate how isoflavones and probiotics influence gut microbiota, immunity, and bone health, and to examine the bioactive and nutritional properties of soybean and sorghum for developing synergistic, functional, value-added probiotic beverages. This review employed a systematic literature search across scientific databases to gather studies on gut microbiota, isoflavones, probiotics, and functional beverages. Relevant findings were analyzed, compared, and synthesized to evaluate bioactive interactions, health impacts, and the functional potential of soybean–sorghum probiotic formulations. The findings highlight the crucial role of gut microbiota in immunity and disease prevention, showing that disruptions contribute to major disorders such as obesity, diabetes, and cardiovascular disease. Isoflavones significantly influence gut microbial balance by promoting beneficial bacteria and inhibiting pathogens, while also supporting bone health through metabolites like equol. Probiotics similarly enhance hormonal, immune, and digestive functions. Combined, probiotics and isoflavones offer synergistic benefits, improving calcium absorption, gut modulation, and metabolic health. Soybean and sorghum bioactives demonstrate strong potential for functional probiotic beverage development. Isoflavones and probiotics synergistically enhance gut microbiota balance, immunity, and bone health, supporting the development of nutritious functional beverages. Their combined metabolic and bioactive effects show strong potential, though further research is required.
Keywords: *Biotransformation, Encapsulation, Fermentation, Isoflavone, Probiotic beverage.*

1. Introduction

Soybean (*Glycine max*) was probably domesticated in China's Huang-Huai Valley, characterized by alkaline soils, approximately 6000–9000 years ago [1]. Soybeans are the only legumes among the four major grain crops grown worldwide, along with maize, rice, and wheat [2]. Due to its numerous advantages, soy protein production and use have become very large in Western countries, especially the US, where it is incorporated into many food products available in grocery stores.

In addition, due to their high protein composition and potential health benefits, soy-based protein foods have been regarded as the primary alternative to animal foods in plant-based diets [3]. Soybean is also known as "poor man's meat" since they have more protein than a non-vegetarian diet. Furthermore, it contains various vitamins, minerals, and bioactive compounds, including isoflavones, which offer several health advantages, such as defense against cancer, osteoporosis, cardiovascular disease, and age-related illnesses [4]. A "Soy Protein Health Claim" stating that consuming 25 g of soy protein (i.e., soy isoflavones) daily may lower the risk of heart disease was also approved by the FDA on October 26, 1999 [5].

Among all leguminous crops, soybeans produce the most isoflavones. They are the only significant salutary source of these compounds. The size and chemical structure of isoflavones is analogous to estrogens, which bind to both the α and β receptors. Because of this, they are frequently referred to as "phytoestrogens." While soybeans contain isoflavones in glycosylated form, their aglycones are

History: Received: 27 October 2025; Revised: 5 December 2025; Accepted: 9 December 2025; Published: 1 January 2026
* Correspondence: deepa.dee1920@gmail.com

responsible for their biologically active nature. When people consume soy products, they convert soy isoflavones into their aglycones using β-glucosidase from enteric bacteria. Isoflavones are part of the larger isoflavonoid family, which also includes rotenoids, pterocarpans, coumestans, 2-arybenzofurans, 3-arylcoumarins, isoflavones, isoflavans, isoflavanone, isoflavonols, isoflav-3-enes, α-methyldeoxybenzoin, and coumaronochromones (Figure 1). The principal isoflavone phytoestrogens are glycitein, daidzein, and genistein. Recent decades have seen strong recommendations for consuming isoflavone-rich functional foods due to their implicit health benefits in fighting aging-related issues, including cardiovascular disease, osteoporosis, hormone-related cancers, and cognitive decline. While isoflavones are present in numerous factory-ground foods, such as cereals, potatoes, fruits, and vegetables, soy-derived foods are the richest source of these compounds in our diet [6].

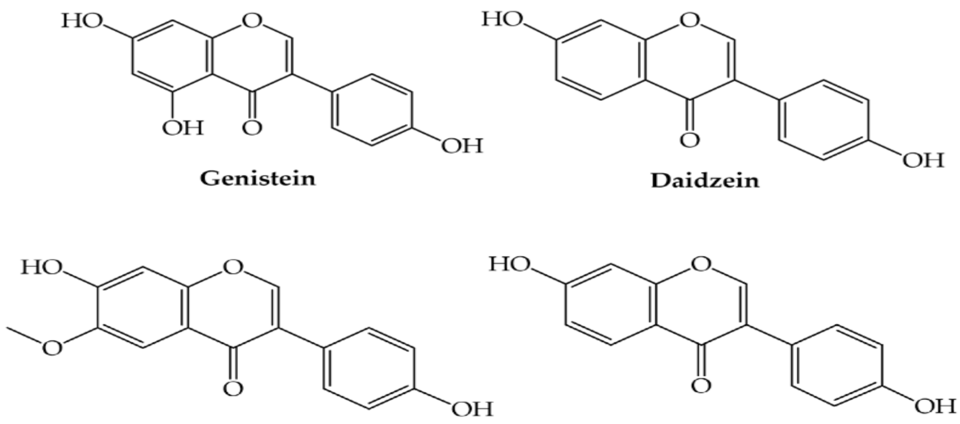

Figure 1.
Examples of isoflavone.
Source: Liga et al. [7].

The majority of countries cultivate sorghum (*Sorghum bicolor* L. Moench), the fifth most cultivated crop, mainly for animal feed. However, due to the cereal's intriguing nutritional makeup, it is possible to employ it significantly to improve the nutritional value of starch-based meals, which are often manufactured from non-wholemeal flours like wheat, rice, and maize. It is feasible to enhance the nutritional appeal of cereal-based human food with sorghum flour, which, in sum, consists of a high proportion of dietary fiber, fat, and protein, along with some micronutrients and a range of bioactive molecules. Sorghum is also gluten-free (GF), making it a very popular ingredient among individuals seeking to enhance the nutritional profile of GF foods. From a production perspective, sorghum is of interest for juice manufacturing. Juice is prepared by mechanical pressing and then purified using hot water at 90°C for a few minutes, generally 10-15 minutes, followed by centrifugation at 6000 RPM for 10 minutes to remove insoluble particles. The fermentable sugars present in the juice are converted into functional carbohydrate molecules, resulting in a highly acceptable functional beverage containing high levels of prebiotic oligosaccharides, which are beneficial to gut health. Sweet sorghum juice is a potential raw material for manufacturing new functional beverages [8].

Today, in the current scenario, these foods are eaten with a better appreciation for their health value. Through fermentation, lactic acid bacteria (LAB) not only change the flavor and texture of foods but also imbue them with a variety of bioactive compounds and signaling molecules that can have positive impacts on human health. The health-promoting bacteria and end products interact with the gut microbiota, at times virtually as an orchestra, all in harmony with each other in maintaining the intestines and the body in good health. The nutritional function of LAB in fermented foods and drinks goes far beyond flavor and preservation. The bacteria break down complex compounds, making them more bioavailable, and the body is thus able to metabolize them more effectively. For example, in milk foods like yogurt and kefir, LAB make protein more digestible and reduce lactose content, thus rendering these foods more acceptable to individuals with lactose intolerance [9].

Probiotic fermentation, especially by lactic acid bacteria, has the ability to convert glycosylated isoflavone forms into more bioavailable aglycones, which are better absorbed by the human body [10, 11]. Such biotransformation can greatly enhance the active isoflavone content and thus optimize their health-promoting activity [12]. Blending these components together in one drink presents a functional food that takes advantage of both, where the probiotic sorghum improves gut health, and the increased bioactivity of isoflavones promotes bone health and menopausal symptom relief [13]. These innovations respond to the growing consumer interest in plant-based functional foods that provide particular health benefits [14].

2. Biochemistry of Isoflavone

Isoflavones are a class of plant-derived polyphenolic compounds (phytoestrogens) found predominantly in legumes, especially soybeans. Their biochemistry involves a unique plant biosynthesis pathway, significant metabolism by human gut microbiota, and a wide array of biological activities mediated primarily by their ability to bind to estrogen receptors [15, 16].

2.1. Biosynthesis in Plants

Isoflavone synthesis is a branch of the general phenylpropanoid pathway. Key steps include: The amino acid L-phenylalanine is converted to 4-coumaroyl-CoA through a series of enzymatic reactions involving phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), and 4-coumarate CoA ligase (4CL) [15, 16]. Chalcone synthase (CHS) and chalcone reductase (CHR) catalyze the condensation of 4-coumaroyl-CoA with three molecules of malonyl-CoA to form the chalcone intermediate liquiritigenin chalcone (for daidzein) or naringenin chalcone (for genistein) [15, 16]. Chalcone isomerase (CHI) facilitates the formation of the flavanone naringenin or liquiritigenin. Aryl migration (committed step): Isoflavone synthase (IFS), a cytochrome P450 enzyme, catalyzes the committed step, involving a 2,3-aryl migration of the B ring to form 2-hydroxyisoflavanone [15, 16]. 2-hydroxyisoflavanone dehydratase (HID) then dehydrates the intermediate to yield the primary isoflavone aglycones: genistein and daidzein (and glycitein). In plants, these aglycones are rapidly converted to glycoside forms (e.g., genistin, daidzin), primarily malonyl or acetyl glucosides, for storage in vacuoles [15, 16].

2.2. Metabolism in Humans

After ingestion of soy foods (mostly as glycosides), human metabolism significantly alters the structure and bioavailability of isoflavones: in the gastrointestinal tract, the glycoside forms are hydrolyzed by intestinal mucosal and bacterial β-glucosidases to release the absorbable aglycone forms (daidzein, genistein, glycitein) [6, 17]. Aglycones are absorbed in the small intestine, reaching peak plasma concentrations in a few hours. They bind to plasma proteins and are distributed to various tissues. In the intestinal cells and liver, the aglycones undergo Phase II metabolism, mainly conjugation with glucuronic acid and, to a lesser extent, sulfation, to form more hydrophilic metabolites [6, 17]. Unabsorbed isoflavones reaching the colon are further metabolized by gut bacteria into more potent metabolites, notably S-equol (from daidzein) and O-desmethylangolensin (O-DMA). The ability to produce equol is a significant individual variable, found in only 30-50% of people, and it affects health outcomes [6, 17]. Isoflavones and their metabolites are primarily excreted in urine and bile, with a half-life of approximately 6-8 hours.

ISSN: 2576-8484
Vol. 10, No. 1: 232-257, 2026
DOI: 10.55214/2576-8484.v10i1.11607

2.3. Biological activities and mechanisms

Isoflavones are termed "phytoestrogens" due to their structural similarity to the human hormone 17β-estradiol. Their bioactivity stems from multiple mechanisms: they bind to both estrogen receptors (ERα and ERβ), with a 20-30-fold greater affinity for ERβ. This selective binding allows them to act as selective estrogen receptor modulators (SERMs), exerting both estrogenic and antiestrogenic effects depending on the tissue and local estrogen levels [15, 16]. Genistein is a known inhibitor of protein tyrosine kinases (PTKs) and DNA topoisomerase II, enzymes involved in cell proliferation and survival signaling pathways. As polyphenols, isoflavones act as antioxidants, scavenging reactive oxygen species (ROS) and inhibiting lipid peroxidation, which may protect against oxidative stress-related diseases [15, 16]. They also interact with other pathways and receptors, such as peroxisome proliferator-activated receptors (PPARs), and influence gene expression related to cell cycle control, apoptosis, and angiogenesis.

3. Isolation, Purification, Concentration, Analysis, and Quality Control of Active Compounds

Natural sources such as plants have a broad spectrum of compounds, but only a handful of them might possess the desired bioactivity or industrial utility [18-20]. Extraction provides a means of selective isolation and enrichment of the active components from inert or unwanted matrix materials. For instance, in the pharmaceutical industry, the Active Pharmaceutical Ingredient (API) needs to be separated from plant material to produce effective drugs. Raw materials usually contain unwanted impurities that may affect the efficacy, stability, or safety of the end product [18-20]. Purification through extraction removes such impurities and concentrates the target compounds to ensure increased potency or activity. This process is especially crucial in extracting essential oils, flavor encapsulation, and nutraceuticals manufacturing.

Extraction is an important step in sample preparation in analytical chemistry to separate analytes from intricate matrices prior to detection and quantification [18-20]. This guarantees precise measurement and quality control in diverse products, ranging from food safety analysis to environmental monitoring. The concentration of target compounds through extraction increases the sensitivity of analytical tools, enabling the detection of materials at very minute concentrations [18-20].

3.1. Solvent Extraction of Isoflavones

Solvent extraction is also the fundamental step of most other extraction techniques, and more accurate or efficient extraction techniques might be constructed by virtue of other equipment or procedures (Figure 2) [19, 20]. Because of the dissolving properties of flavonoids, alcohol or alcohol–water mixtures are frequently used as solvents in extraction [19, 20]. The solvent is usually an organic solvent or a blend of an organic solvent and water. Based on the extracted substance, organic solvents of varying polarities can be used, including n-hexane, petroleum ether, acetone, methanol, ethanol, chloroform, etc [19, 20]. Occasionally, auxiliary methods such as heating, ultrasonic, or reflux extraction are also employed to enhance the rate of extraction, and other auxiliary pH regulators like formic acid are added in order not to destroy the pH-sensitive substances [21].

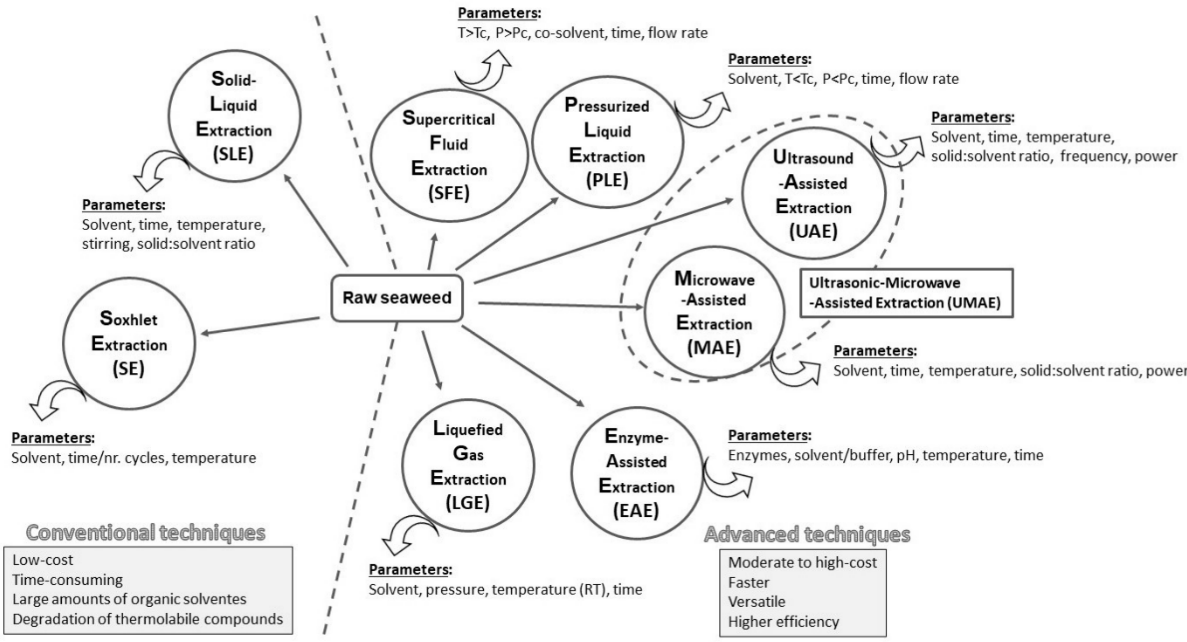

Figure 2.
Types of Solvent Extraction.
Source: Quitério et al. [22].

Extraction methods are categorized into two broad categories: traditional methods comprise percolation, maceration, Soxhlet extraction, and non-traditional methods like microwave-assisted extraction, ultrasound-assisted extraction, and enzyme-assisted extraction [19, 20]. Plants extract the highest bioactive compounds by different methods, primarily using various aqueous-organic solvents. Non-traditional methods generally possess higher efficiency compared to traditional methods because they have shorter extraction times, lower costs, and produce compounds of remarkable purity [19, 20]. Both methods have several drawbacks, including the toxicity of classical solvents (e.g., hexane and methanol), thermal instability, low recovery of bioactive compounds from classical solvents, and potential impacts on the chemical structures of the compounds via various pathways [23].

A single extraction method may be insufficient, whereas the combination of techniques has a better yield of compounds, which is attributed to the specification, nature, and structural properties of the plants from which it is obtained. Improved yield, enhanced product quality, and lower environmental footprint can all be obtained through the application of newly established green extraction methods such as UAE, EAE, MAE, etc [24].

3.2. Maceration

Maceration is a traditional and straightforward method of extraction where the plant raw material is prepared and soaked in a coarse or powdered infusion in a solvent of choice at room temperature for a minimum of three days with regular agitation [25]. The extracted solution is filtered either through a net or a sieve with very small or permeable holes. Subsequently, the liquid that is extracted is purified using either decantation or the filtration method once it has settled. Maceration is best performed in a closed or stoppered vessel to reduce the loss of solvents by evaporation [25].

Maceration is the application of low-boiling, volatile organic solvents, such as petroleum ether and tetrachloromethane, to induce a more selective and efficient mass transfer of plant compounds through the application of different solvents, temperatures, and combinations of stirring to achieve the desired extract [26]. The organic solvent is recovered via vacuum distillation and other techniques to produce a pasty extract, which is employed to produce the absolute. This method offers advantages such as an easy procedure, straightforward equipment handling, a high efficiency rate in extraction, and the capacity to select solvents based on the desired constituents or compounds present [26].

This has been optimized through agitation to improve compound diffusion into the solvent and thereby raise the temperatures to aid dissolution, decrease the solvent viscosity, and decrease the size of the solid material particles to improve mass transfer and thereby the rate of extraction. However, this type of extraction has limitations such as long extraction times, a high amount of vegetal mass and solvents, and low yield [27].

3.3. Ultrasound-Assisted Extraction

The fundamental principle of ultrasonic extraction technology is to utilize ultrasonic cavitation, mechanical, and thermal effects to promote the release, diffusion, and dissolution of active ingredients inside the cell, thereby improving the extraction efficiency (Figure 3) [28]. When a medium is subjected to high-energy ultrasonic waves, the medium is disintegrated into many small cavities, which close up at once, creating an instant pressure of several thousand atmospheres. This process is known as cavitation [28]. The collapse of minute bubbles in cavitation creates enormous pressure, bursting cell walls of plants and the whole organism completely in an instant, reducing fragmentation time. At the same time, ultrasound-induced vibration facilitates the release, diffusion, and dissolution of intracellular content, thereby greatly improving extraction efficiency [28].

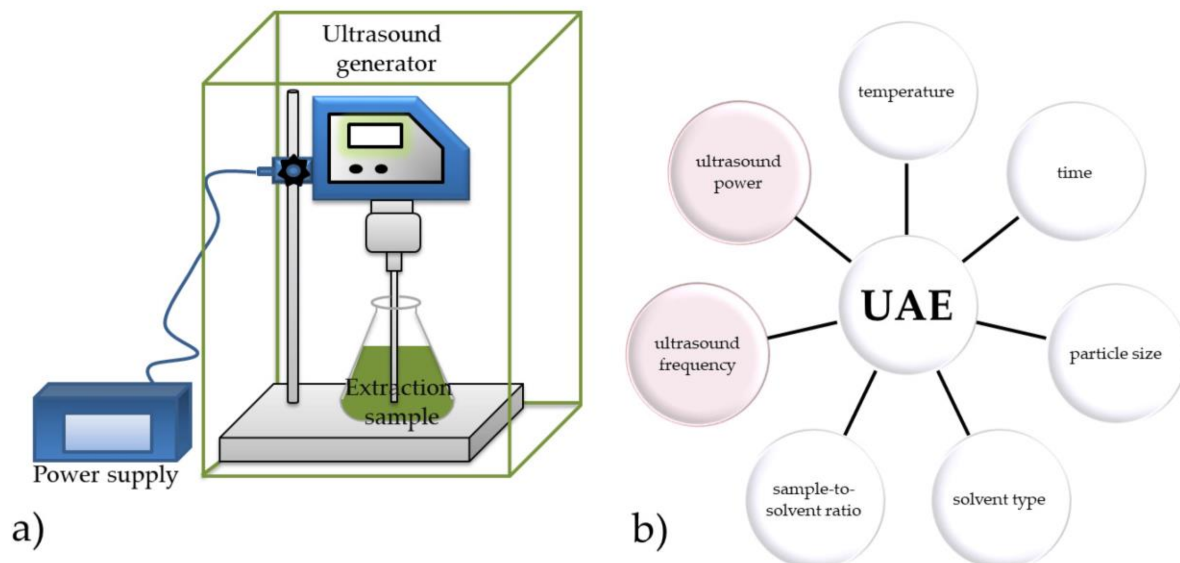

Figure 3.
Figure (a) Schematic Illustration of UAE Technique, Figure (b) Factors facilitating effective UAE.
Source: Liga et al. [7].

For effective extraction of bioactive compounds, ultrasound allows a high yield of extraction with relatively lower temperature and avoids the degradation of such compounds and the resultant quality loss of the end extract. The integration of ultrasound with natural solvent application is consistent with the concept of an eco-friendly and green extraction process [29]. Ultrasound refers to sound waves or acoustic energy higher than what is audible to the human ear (i.e., 20 kHz and above). Ultrasound has numerous applications and facets within food science. Due to its contribution toward environmental sustainability, ultrasonication is used largely at the industrial level and is termed a "green unique technology." Ultrasonication treatment varies from 20 to 100 kilohertz (kHz), and the power ranges from 10 to 1000 W/cm^2. If the power range is from 20-100 kHz, then it is entitled as ultrasound. It is of two types: high-frequency ranges from 100 kHz to 1 MHz, and diagnostic ranges from 1-10 MHz, based on the frequency used for the technique. Polar solvents such as water and non-polar solvents such as ethanol and acetone are utilized using ultrasonic-assisted extraction (UAE), influencing the volume that can be extracted [30].

For UAE processing, low frequency at high intensity produces intense shear and mechanical force assisting in extraction, and higher frequency sound waves produce numerous reactive radicals. Certain works were examined for evaluating the volume and quality of extraction based on varying frequencies, but the majority of these proposed that the operation must occur at a fixed frequency [30]. Power utilized for extraction depends on the raw material being processed and varies from 20-700 W. The volume extracted increases with power and then decreases at a specific threshold. Additionally, higher power results in increased mechanical vibration, which enhances the contact area between the solvent and tissue matter, leading to greater solvent penetration and higher extraction efficiency. Power, time, and solvent parameters are critical factors in ultrasonic-assisted extraction (UAE) [30].

3.4. Microwave-Assisted Extraction

Microwave-assisted extraction technique, commonly applied for trace analysis of organic substances in liquid and solid samples (Figure 4), is solvent extraction-based. MAE exploits the internal heat effect of the microwave (thermal stress ruptures the cells; the material and solvent molecules frictionally collide continuously with the change in alternating electromagnetic fields, and the level of cell breaking rises). It is favored by low liquid consumption, high efficiency in extraction, strong selectivity, low contamination, and even heating, which prevents the gelatinization and agglomeration of the drug materials [31].

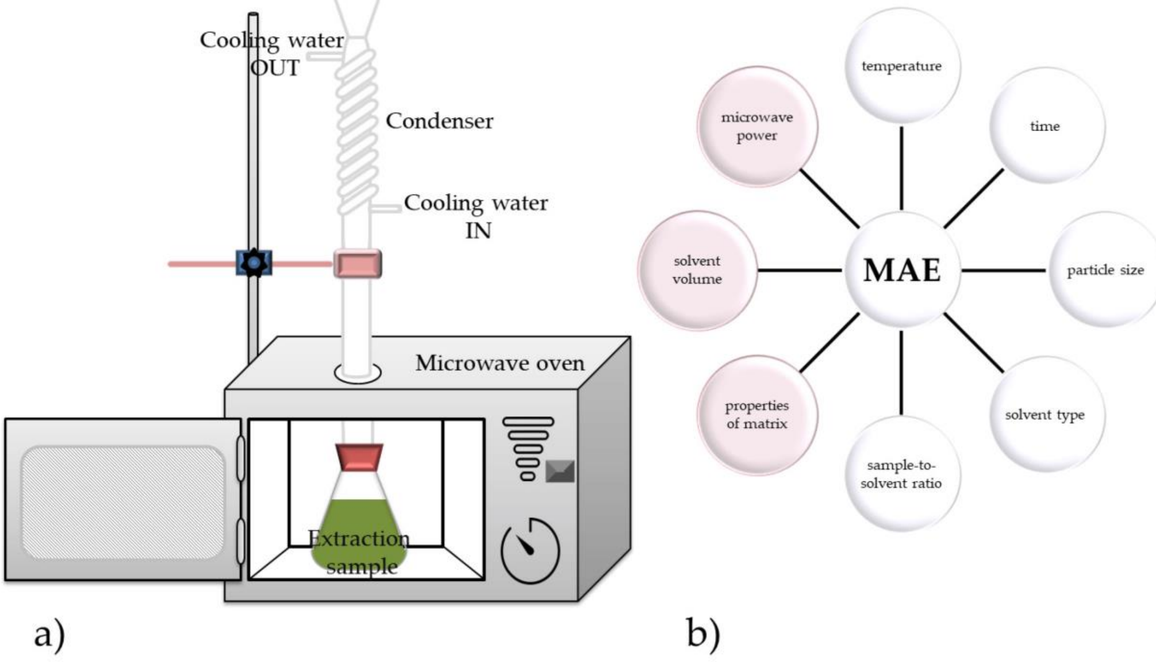

Figure 4.
Figure (a) Schematic Illustration of MAE Technique, Figure (b) Factors facilitating effective MAE.
Source: Liga et al. [7].

In microwave-assisted extraction (MAE), the matrix is heated using microwaves. Their frequency ranges from 300 MHz to 300 GHz. The Electric and magnetic fields of microwaves are perpendicular to one another. The electric field induces heating through ionic conduction and dipole rotation. Depending upon their dielectric constants, components are capable of absorbing microwave radiation. The microwave radiation facilitates cell disruption, making the liquid penetrate into the plant matrix. On the other hand, plant material enters the solution from the cell's exterior surface. The efficiency of microwave-assisted extraction is based on: the nature and quantity of solvent, extraction time, microwave power, operating temperature, and matrix properties. All the above should be considered in process optimization [32].

Compared to the UAE in optimal experimental conditions, MAE is eight times faster and consumes six times less solvent, and it is also more effective than a shaking water bath. Generally, compared with traditional methods, MAE reduces solvent consumption and extraction time; however, microwave heating may cause oxidation and degradation of target compounds. MAE is primarily limited to small polar molecules and is not suitable for thermosensitive compounds [12].

3.5. Enzyme-Assisted Extraction (EAE)

Enzyme-assisted extraction is a pretreatment process that uses specific enzymes to lyse the cell wall of the source material in order to increase recovery yield (Figure 5). It can be integrated with other operations to further maximize the overall recovery of bioactive compounds from other biomaterials [7].

Further, EAE also has some advantages, such as utilizing the whole plant material, soft reaction conditions, fewer processing steps, the isolation of high-quality bioactive compounds, and bioavailability [33].

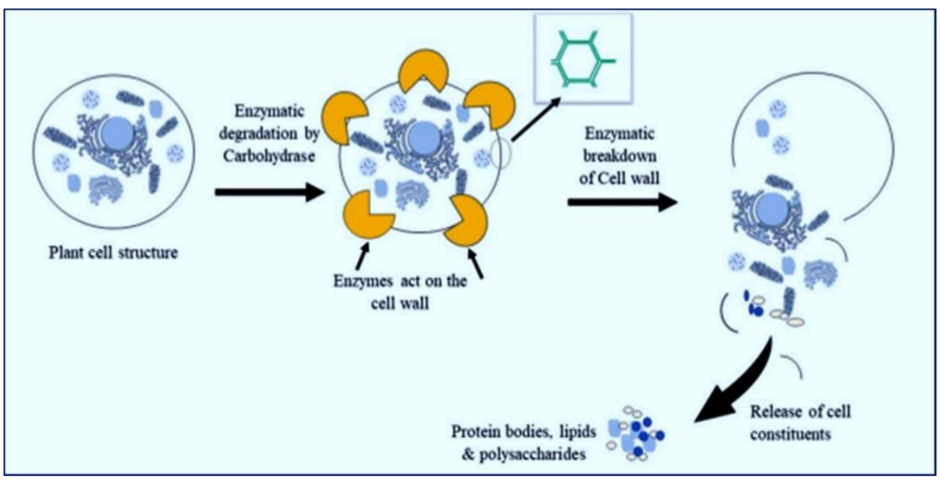

Figure 5.
Degradation of the plant cell wall in EAE.
Source: Anyiam et al. [34].

The EAE is based on the use of enzymes that catalyze the cleavage of covalent bonds under water conditions. This process breaks cell structures, and the permeability of the material is enhanced. The enzyme-aided extraction can be a stand-alone process or a pre-treatment before normal extractions [35]. Enzymes are highly specific under optimal conditions. Particle size, time, pH, and temperature need to be taken into consideration. Enzymatic reactions proceed effectively at relatively low temperatures and moderate pH levels, typically within a short time frame (up to a few hours). These processes generally do not require costly equipment [35]. The time and gentle conditions enable minimizing degradation or isomerization of active molecules. In most instances, this enzymatic pretreatment is said to have a positive or even strongly optimistic effect on extraction efficiency. The EAE technique has been employed for the separation of low molecular compounds (e.g., oils and fatty

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 10, No. 1: 232-257, 2026
DOI: 10.55214/2576-8484.v10i1.11607

acids, polyphenols and phenols, essential oils, sugars, di- and triterpenes, vitamins) and macromolecules (polysaccharides, proteins) [35].

Two types of extraction methods that use enzymes are enzyme-assisted aqueous extraction (EAAE) and enzyme-assisted cold pressing (EACP). An example that demonstrates their effectiveness is the use of enzymes in microwave processing. This combined method significantly improved the extraction yield of phenolic compounds. It outperformed the lower recovery yields obtained with conventional solvent extraction using water. This improvement is attributed to the high extraction temperatures and the rapid heating methods [36]. Further, compared to other extraction methods that do not involve enzymes in the process, the pectin yields from some wastes were drastically higher when enzymes were applied, followed by ultrasound treatment. It is challenging, however, to select the correct enzymes. It depends on several factors, including the plant matrix, target compounds, and the conditions required for extraction [36].

3.6. Supercritical Liquid Extraction

When the temperature of the substance is raised above its critical temperature T_c) and the pressure above its critical pressure (P_c), a new phase, distinct from the solid, liquid, and gaseous phases, is produced, and the new fluid region is referred to as "supercritical fluid (SF)" (Figure 6). The physicochemical properties of SF are between those of liquids and those of gases. This characteristic makes SF a better solvent [37]. The primary underlying objective of supercritical extraction is the acquisition of extracts with high-quality properties, specifically enhanced purity and increased concentration of the compounds of interest. The process induces a selective potential for the extraction of specific components of interest, thereby enabling the intentional separation of desired components from undesired substances, and at the same time offering the prospect of enhancing the compositional precision of the resulting extract. Supercritical extraction has several applications in various industries such as pharmaceuticals, food, cosmetics, and beverages [38].

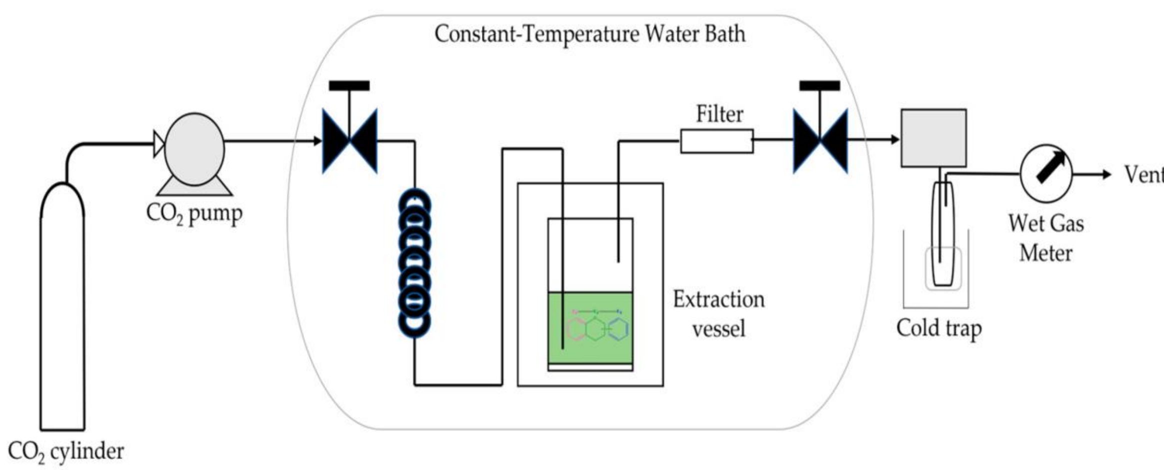

Figure 6.
Schematic illustration of the SLE (Supercritical Liquid Extraction) Technique.
Source: Liga et al. [7].

Supercritical fluids have the benefit of being able to recycle since reversal of the temperature and pressure change allows the recovery of a specific substance from a food matrix. Supercritical fluid extraction permits the acquisition of solvent-free extracts, and the process is quicker compared to the utilization of traditional organic solvents, without leaving toxic residues as a key technology in "green chemistry." Besides, it involves a high price owing to the initial equipment and maintenance investment.

DOI: 10.55214/2576-8484.v10i1.11607

Solvent compression is subject to sophisticated recirculation schemes to minimize energy expenditures. It can dissolve some polar substances, and the employment of modifiers will modify the polarity of CO_2, so a downstream separation process needs to be incorporated into the obtained extract [39].

Substances that have low molecular weights and are less volatile but more polar can be efficiently separated by this method. Although carbon dioxide is a greenhouse gas, it may still be recycled without harming nature if handled properly, extracted, processed, and released back into the environment. Moreover, SCFs have good thermal transport properties, i.e., they can exchange heat. Unlike existing technologies that make use of toxic materials or release potent greenhouse gases, they do not have a significant negative environmental influence when used as heat transfer fluids [40].

4. Probiotic Sorghum Beverage

Nowadays, the worldwide market for probiotic foods is greater than ten times that for probiotic supplements, indicating a consumer preference for intake through food and beverages versus nutraceuticals. Fruit and cereal fermentations have considerable promise for the production of new probiotic drinks. It is important to note that fruits and cereals have many nutritional and health-supportive benefits. When synergistically blended with probiotics, they can even further augment a variety of biological activities. Thus, the development of non-dairy fermented drinks with the inclusion of probiotics is a rising trend in the functional food industry [41].

The impact of lactic acid fermentation on the nutritional quality of fermented cereal- or pseudocereal-based drinks is, however, variable, although data from literature show an improvement in the characteristics of such products. You can bypass obstacles by selecting the right LAB (lactic acid bacteria) cultures for the right formulation of plant drinks made out of cereals or pseudocereals. Besides, cereal- or pseudocereal-based drinks are free from the proteins, lactose, and cholesterol present in milk. Plant drinks made out of fermented cereals or pseudocereals have many proteins, fiber, vitamins, and minerals as well. Therefore, these drinks cannot only be a fine selection of food for individuals suffering from celiac disease, milk protein allergy, or lactose intolerance, but also an innovative, eco-friendly option for everyone else [42].

Sorghum (*Sorghum bicolor* (L.) Moench) is a whole-grain cereal food that, similar to other whole grains, promotes health and moderate disease conditions. To show the health benefits of sorghum, research has explored its nutrients and bioactive compounds, particularly phytochemicals, starches, non-starch polysaccharides, proteins, and lipids. Such grain components have been associated with actions on energy balance, regulation of satiety, glycemic control, and lipid control. Positive effects on antioxidant and anti-inflammatory activities, as well as on modulation of gut microbiota, have also been described. Overall, all these sorghum grain components may positively affect metabolic health and disease markers, such as body weight, and thus underscore the potential of sorghum as a disease prevention agent against chronic diseases [43].

Fermentation is rated as the best method of treatment, followed by the combination of other treatments like soaking, germination, and nixtamalization (Figure 7). Thus, sorghum may be applied in numerous food items such as bakery products, extruded items, beverages, and porridge. Sorghum-ogi is regarded as one of the probiotic foods, which is derived from wet-milled fermented sorghum. Its texture is smooth, creamy, free-flowing, and it is a thin porridge [44].

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 10, No. 1: 232-257, 2026
DOI: 10.55214/2576-8484.v10i1.11607

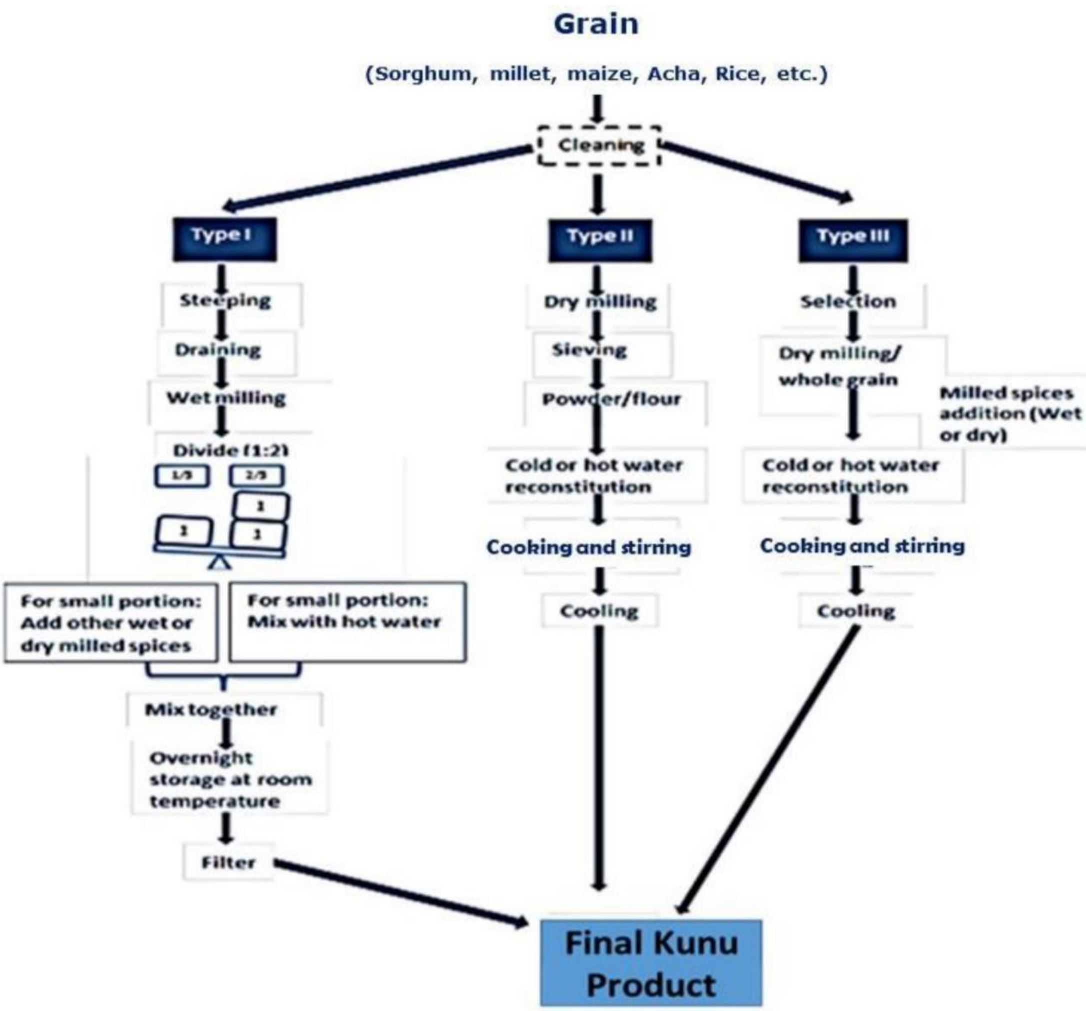

Figure 7.
Flowchart of probiotic beverage from grains.
Source: Ndukwe et al. [45].

4.1. Soaking or Steeping

Soaking is an elementary process that involves the diffusion of water into grains via the hilum until equilibrium moisture content is attained. It tends to facilitate dehulling, milling, germination, cooking, and enzyme activation [46]. Sorghum grains are usually soaked before the start of the process. Soaking for a period of time results in physical and chemical alterations in the seeds. When soaked, water enters the kernel, causing the breaking of the cell walls of the grain; therefore, it softens and swells the physical structure, due to which the water-soluble constituents are liberated. However, a study determined that soaking can raise or improve the overall flavonoid content present in sorghum but did not alter total phenolic content, condensed tannin content, or antioxidant activity. Here, we can simply understand that soaking can decrease water-soluble components in sorghum [47].

4.2. Germination

Germination of grains is an inexpensive and uncomplicated method to enrich the nutrition of pseudocereal and cereal kernels. Germination has been extensively employed in enhancing cereal quality, making the kernel structure softer and increasing bioactive contents in cereal seeds. For germination, the seeds need to be sterilized and soaked in water with oxygen and subjected to sprouting under controlled temperature, relative humidity, and light/dark regimes, where endogenous enzymes are synthesized and released. Moreover, soaking of grains should be carried out properly since the seed embryo needs to continue its metabolic activities so that the synthesis and release of endogenous enzymes take place efficiently [48].

In some of the sorghum samples, germination reduces the protein content (12–21% on average) but enhances protein solubility and digestibility. This is because germination causes high proteolytic activity of storage proteins and improves their bioavailability and amino acid content, such as lysine, valine, phenylalanine, methionine, and tryptophan. Germination has reduced tannin, phytic acid, and oxalate content in sorghum. The reduction of tannin content was attributed to hydrogen bonding and nonpolar hydrophobic interactions between proteins and tannin [49].

4.3. Thermal Processing

Thermal processing is a critical process in the food processing sector to ensure microbiological safety, destroy anti-nutritional factors, and impart the desired color and flavor to foods [50]. Thermal processing may decrease the level of tannins and phenolic compounds, which are anti-nutritional agents. Hence, the biological activity of phenolic compounds will also be influenced by thermal processing, as they are heat-sensitive [51].

Steaming is one of the conventional methods frequently utilized preceding and succeeding other processes like germination, microwave processing, and cooking. Millets such as finger millet, barnyard millet, sorghum, and other legumes and grains frequently undergo steaming as part of processing. Steaming entails the exposure of millet grains to steam, leading to the use of heat without contact with water. Steaming is useful in the gelatinization of starches in millet grains, thus enhancing digestibility and minimizing cooking time. Gelatinized starches increase the texture to be softer and enhance the palatability of millet foods overall. Steaming also reduces resistant starch content and retains water-soluble vitamins and minerals, maintaining millets' nutritional quality. Steaming also produces very little protein denaturation, improves protein digestibility by disrupting complex protein structures, and leads to enhanced digestibility and production of nutritious and good-value-added millet food products [52, 53].

Microwaving sorghum grain is also capable of enhancing the quality of SF in terms of colour, texture, and sensory attributes. For instance, microwaving sorghum flour weighing 100 g in a microwave oven for a range of between 36 and 90 kilojoules was reported to improve the flour's physical properties. Other than improving the quality of flour, microwaving also increases the shelf life of flour even in hot storage. Dry heat treatment of sorghum grain can be utilized in conjunction with particle size distribution. Some functional characteristics, such as water absorption ability and an increase in fat and fiber, were affected by the approximately determined composition of flour, whereas moisture, protein, solubility index, ash or mineral content, water holding capacity, and foam formation were not affected by it [54].

Heat treatment can modify the structure of proteins, prolong their molecular structure, and enhance the surface contact between digestive enzymes and proteins. The right temperature can enhance the extent of gelatinization and hardness of starch, thereby improving feed digestibility. However, excessive temperatures can initiate the Maillard reaction in amino acids, which can increase hardness. Additionally, elevated temperatures can interfere with noncovalent protein-protein interactions, promoting structural modifications and the formation of disulfide linkages between β-kafirin and γ-kafirin that encapsulate α-kafirin and reveal the remains or sediments of hydrophobic amino acids present in the proteins, ultimately resulting in lower or poor solubility of the protein. Conversely, low temperatures can reduce the extent of starch gelatinization, leading to low feed viscosity, poor pellet strength, and reduced protein digestibility [55].

4.4. Nixtamalization

Nixtamalization, as traditionally defined, is a heat process that involves cooking corn kernels in a water-alkaline treating agent or water-calcium material solution for the overall purpose of pericarp and endosperm softening and easy grindability. While corn remains the primary crop treated with nixtamalization, other grains such as sorghum, wheat, rice, oats, barley, amaranth, beans, soybeans, potatoes, and chia have also been subjected to this process. Although nixtimalization has been utilized for centuries, it has only been scientifically investigated since the 1940s. Its significance to human nutrition is well established, as it enhances the bioavailability of niacin, lysine, and tryptophan, along with calcium content, which has helped to prevent pellagra, rickets, and osteoporosis in certain American populations. Beyond these nutritional benefits, nixtamalization also affects the starch and pericarp tissue, which together constitute 80.5–92.9% of the grain weight. These effects influence processing handling, as well as the quality and sensory characteristics of nixtamalized corn products, including texture [56].

Nixtamalized maize is easier to grind, richer in nutrients, superior in flavor and aroma, and less likely to produce mycotoxins. The kernels of maize are typically cooked or boiled in water with lime (calcium hydroxide) and steeped overnight. A crucial process is the diffusion of water and calcium ions into the maize kernel during the alkaline treatment (steeping and cooking). The high pH and elevated cooking temperatures facilitate the absorption of water, soften the endosperm, and remove the pericarp during the nixtamalization process. The excess lime and loosened pericarp are washed away from the alkaline-cooked kernels (nixtamal) by water after draining the steeping liquid (nejayote). Sanchez-Reséndiz states that some nixtamalization conditions involve very long boiling times at high temperatures, followed by extended steeping times at hot temperatures. According to various research, enriching soybean flours can reduce dry matter losses, indicating that consumers of mixes made from soy flour can benefit from products with greater nutritional value than those produced solely from nixtamalized maize flour [57].

4.5. Wet Milling

Milling is the most industrially applicable and most widely used method for separation. Milling includes tempering and grinding cereal grains into flour and subsequently sifting the flour to isolate the bran, germ, and endosperm. Following milling, mill streams can be blended to produce flour with particular physicochemical, functional, or nutritional characteristics. The milling process can be adjusted to produce distinct fractions with different particle sizes. The fractions separated from milling can be analyzed for their starch, protein, ash, fiber, and phytochemical content, among other traits like particle size [58].

Milling refers to grinding, crushing, or pulverizing grains into fine fragments to maximize the yield of endosperm flour. Sorghum kernel size is comparable to wheat, and the sorghum pericarp is friable compared to other cereals. Clean sorghum grain may be soaked in water for approximately 2 days, and thereafter crushed to relax the embryo. The starch/protein suspension is then centrifuged to yield the protein separated from the starch. The isolated starch is washed, and either dried for food preparation or hydrolyzed to produce glucose syrup [59].

Milling of sorghum is also feasible using traditional wheat milling methods with minor alterations in accordance with the cultivar characteristics, as there are substantial physical and chemical variations between the cultivars of sorghum, i.e., thickness, hardness, and size of the pericarp [58].

Grain fractionation processing faces several problems, such as food wastage, enormous by-products, and co-processing conditions in wet milling, apart from the detrimental effects on food security and the environment. These processing limitations are factors that need to be addressed in a new concept to make the grain processing industry more sustainable and economically viable. The model to be proposed must be able to incorporate circularity in grain and pulse milling and fractionation by ensuring integration between processes, equipment versatility, and coordination between manufacturing and facility environments [60].

4.6. Fermentation

Fermentation is the oldest biotechnology, wherein a metabolic process is conducted without oxygen involvement. Fermentation is among the food processing techniques that enhance nutrient contents and sensorial properties, possibly reducing or eliminating pathogenic microorganisms and natural toxins. Fermentation enhances the nutritional quality of foods and drinks. Based on the research of certain authors, fermentation increases the essential amino acids in fermented food and drink products. Lactic acid bacteria and yeast can be employed to enrich protein levels in fermented foods and drinks made from cereals [61].

Through food fermentation, the bioavailability of nutrients is enhanced, which in turn provokes probiotic and prebiotic activity, thus increasing the nutritional value and health benefits of the food in question. To achieve this effect, the bacteria must survive gastric transit so that they reach the site of action. Microorganisms need to survive in the intestinal tract to carry out their functions, some of which include gut microbiota modifications and fermentation, among others. Many research studies conducted with putative probiotic microorganisms, including Lactobacillus, Bifidobacterium, Saccharomyces, Enterococcus, Streptococcus, Pediococcus, Leuconostoc, Bacillus, and Escherichia coli, have shown that microorganisms can reach the gastrointestinal tract [62].

Lactic acid fermentation remains the preferred method for food processing due to its ability to preserve foods, enhance shelf life, and improve flavor. LAB (Lactic Acid Bacteria)-fermented cereals have proven to be effective delivery vehicles for probiotic bacteria. Sorghum was utilized as a carrier for targeted probiotic strains and is a climate-tolerant crop, as well as a low-cost commodity accessible to most Southern African households. The four targeted probiotics were controlled for fermentation to assess amylase activity, microbial load, pH, and lactic acid content in the presence or absence of glucose. The targeted strains were also tested for inhibitory activity against pathogenic bacteria. Throughout the fermentation process, all targeted Lactobacillus strains grew well, reaching over 6 log10 cfu/mL after 24 hours, regardless of the presence or absence of sugar. However, fermentation without the addition of glucose resulted in a minimal decrease in pH, ranging from 4.0 to 4.4 after 24 hours of incubation [63].

But there is considerable interest in the development of other non-dairy food matrices for probiotic delivery. This is being fueled by an emerging consumer interest in plant-based alternatives for treating conditions such as lactose intolerance, cholesterol issues, and milk protein allergic reactions. Over a span of several years, probiotic organisms have been successfully used along with other food matrices, such as cereals, as a novel way of transporting beneficial and functional microorganisms. Lactic acid bacteria are recognized as an important group of probiotic microorganisms, with many strains primarily found in the gastrointestinal tract. These bacteria are resistant to extreme environmental forces such as low pH levels, bile salts, natural growth inhibitors, and interactions with other microbes [64].

Although fermented foods are generally safe owing to the antimicrobial action of LAB and other fermenting microorganisms, safety risks still exist owing to the artisanal nature of the production process. These include microbial contamination, the production of toxins, and variability in fermentation conditions. Fermented foods involving spontaneous fermentations have typical risks of microbial contamination and the requirement of adequate control of fermentation conditions, such as time, temperature, and pH. Although such similarities exist, the nature of the influence of these risks may vary depending on the specific fermentation practices and raw materials involved. Furthermore, poor handling and the use of unclean packaging materials also present microbial hazards after processing. Products based on cereals such as millet, sorghum, and maize are most vulnerable to aflatoxin contamination. This can be related to the nutrient content of these foods, as well as their high humidity levels compared to other foods, and poor storage. Overall, even though fermentation does offer protective factors in the form of the formation of health-promoting microbes, there are also safety concerns that are multifaceted and require consideration of the exact fermentation practices as well as the raw materials employed in the process [65].

4.7. Techniques of Incorporation of isoflavones into Probiotic Beverages

Food addition may vary from the inclusion of macro- and micronutrients for product fortification to adding functional ingredients such as prebiotics, probiotics, and bioactives to provide greater health advantages (Figure 8) [66, 67]. The main objectives are usually to combat deficiencies in nutrients, enhance disease protection, increase shelf life, and respond to changing consumer needs for more convenient and healthier food products [14]. The addition of isoflavones to probiotic-fortified foods, such as fermented drinks, is particularly promising for enhancing health benefits by increasing the bioactivity and bioavailability of these substances. The incorporation of isoflavones primarily depends on the metabolic activity of probiotic microorganisms, especially Lactic Acid Bacteria (LAB) [68].

5. Significance of Incorporating Functional Ingredients into Value-Added Products

5.1. Improving The Nutritional Condition and Rectifying Deficiencies

Fortification refers to the process of enriching foods with micronutrients such as vitamins and minerals. Fortification dates back centuries in the history of safely helping to prevent serious nutrient deficiencies worldwide [69]. In the U.S., for example, fortification efforts have greatly helped many people maintain adequate levels of micronutrients [70]. Addition of nutrient-rich foods and key micronutrients enhances total body well-being by supporting the immune system, facilitating better skin health, efficient digestion, and energy production [71]. These supplements are important for achieving proper nutrient consumption. Some of the principal nutrients, such as dietary fibre, vitamin D, calcium, iron, and potassium, tend not to be taken sufficiently by people. Consumption of these nutrients is more likely to prevent conditions like osteoporosis, anaemia, and hypertension [71].

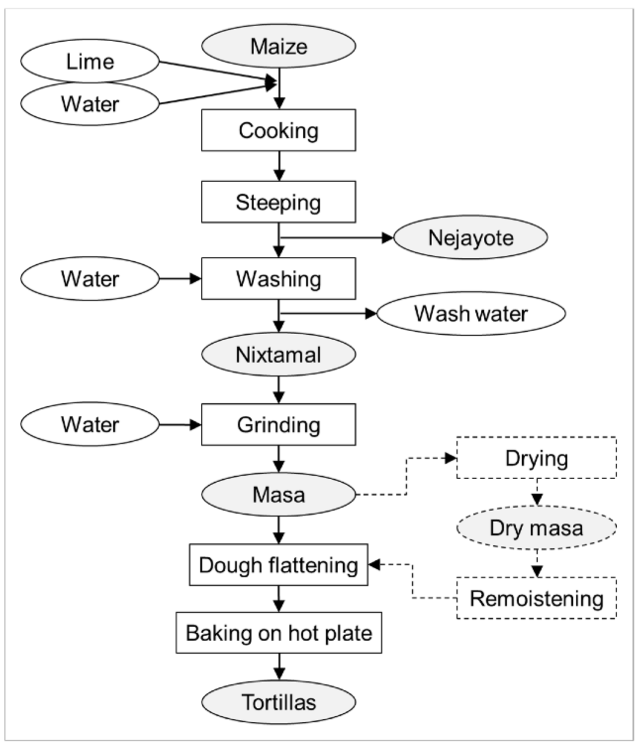

Figure 8.
Flowchart of probiotic beverage from grains.
Source: Schaarschmidt and Fauhl-Hassek [72].

ISSN: 2576-8484
Vol. 10, No. 1: 232-257, 2026
DOI: 10.55214/2576-8484.v10i1.11607

5.2. Preventing and Treating Disease

Proper nutrition, assisted by supplements of useful molecules, may prevent numerous conditions. For instance, a diet high in digestive fibre may reduce blood glucose and lipoproteins and diminish calorie consumption [73]. The incorporation of functional components in foods is supposed to enable individuals to remain healthy, not fall ill, and live comfortably [14].

Foods fortified with unique ingredients such as bioactives and probiotics have the potential to offer beneficial health aspects beyond normal nutrition [66, 74, 75]. For instance, the addition of phenolic compounds to dairy products improves the nutritional value and fulfills consumers' desires for functional foods [75]. Daily consumption of these foods helps to provide energy, boost the immune system, and assist the body against long-standing disease conditions [71, 76].

5.3. Meeting Consumer Demands and Market Trends

Consumers now more than ever desire products with health benefits, such as omega-3s and calcium. The food sector is responding by creating new products with these key nutrients [77]. "Food synergy" is the term used to describe how the many elements of food interact. The theory suggests that the overall benefit of many nutrients and chemicals can add up to more than the benefit of each component. A diet of varying nutrients supports such a theory [78]. Using wholesome nutrients such as whole grains in models of nutrient profiling makes it easier to harmonize diet products and diet recommendations. The latter facilitates healthier consumption patterns on a global level [79].

6. Techniques of Incorporation of Isoflavones into Sorghum-Based Beverages

Incorporation technology, including encapsulation, nanoemulsion, liposome entrapment, and microencapsulation, is essential for the optimization of the functionality, stability, and bioavailability of active ingredients in foods. It allows the controlled incorporation of sensitive compounds like vitamins, probiotics, enzymes, antioxidants, flavorings, and essential oils into food systems without a loss of their activity during processing, storage, or digestion. Encapsulation prevents bioactive compounds from environmental stresses such as heat, light, oxygen, and pH, and hence preserves their nutritional and sensory attributes. In addition, incorporation technologies provide the ability to release ingredients in controlled amounts at specific locations in the gastrointestinal tract, enhancing their absorption and therapeutic effects [80].

These techniques also improve product stability, cover unwanted flavors or aromas, and facilitate the enrichment of foods with functional or therapeutic ingredients. Incorporation technology has been extensively used in the design of functional drinks, milk, bakery foods, and nutraceuticals, and hence it is a major innovation for manufacturing next-generation health foods. Overall, incorporation technology fills the gap between food science and nutrition by ensuring efficient delivery of the active compounds while preserving product quality, consumer acceptability, and long shelf life.

6.1. Encapsulation

In the 1970s, encapsulation technology was applied in the food industry, starting with food additives, ingredients, health foods, and probiotics (Figure 9). Encapsulation technology has been extensively applied to improve the stability, specificity, and bioavailability of the most important food ingredients. In addition, it also has a crucial function in enhancing product quality and minimizing production costs. Encapsulation technology refers to a group of techniques that utilize encapsulation to enclose various substances with the purpose of enhancing their solubility, polarity, and other characteristics or improving their stability against external factors such as light, heat, and oxidation. The material used to trap the bioactive or required compound to enhance its bioavailability and delivery is known as the core material, while the material used in the encapsulation process is known as the wall material [81].

Encapsulation technologies can be divided into chemical and physical technologies. Release characteristics, encapsulation rate, and efficacy changes are also used as significant indicators to assess the efficacy of encapsulation [81]. Genistein is a widely recognized isoflavone; however, due to its poor solubility and low oral bioavailability, carrier systems must be used to overcome these drawbacks [82].

Nanoencapsulation is an essential process that stabilizes and improves the bioavailability of sensitive bioactive compounds in different applications, particularly in the food sector. Through the encapsulation of such compounds in protective nanocapsules it prevents degradation while enhancing their functionality. The nanoencapsulation of active compounds enables the enhancement of their biofunctional properties, which can be beneficial in the food sector. It should be noted that, in order to execute this procedure, there is no common method, as every compound or food possesses a unique molecular structure. Owing to structural diversity, foods possess unique properties from one another, which restricts the standardization of a common process for all foods [83].

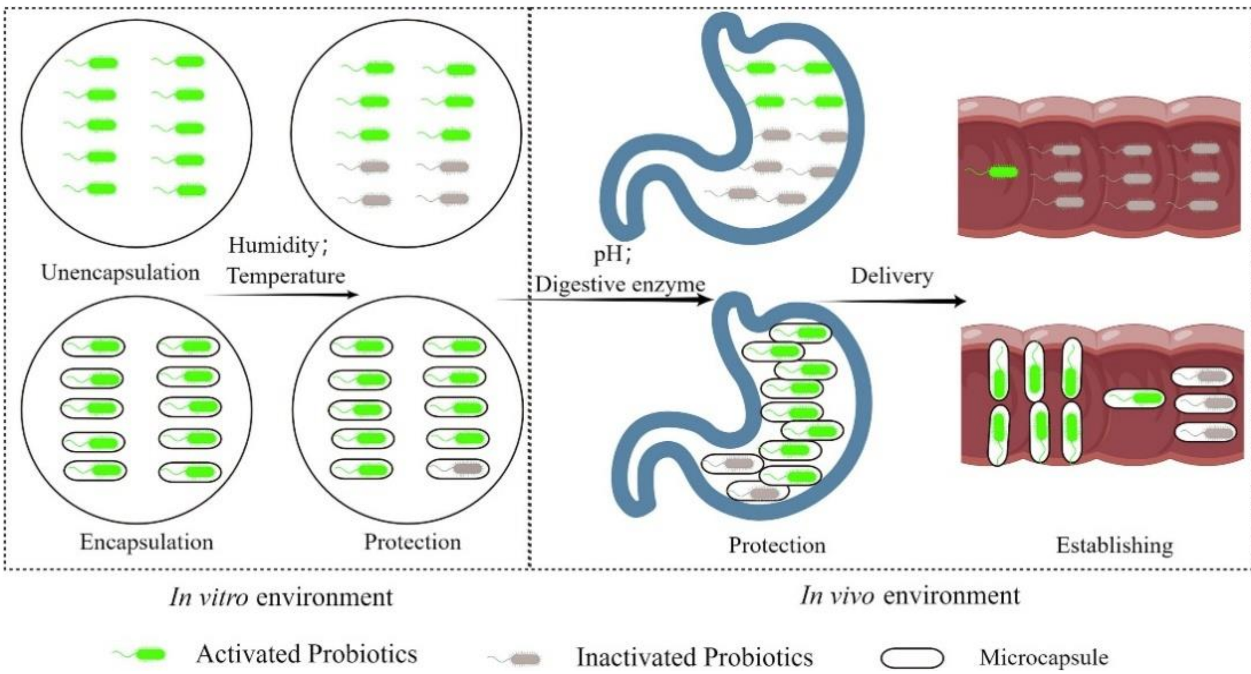

Figure 9.
Stability and protection of the encapsulation in both in vitro and in vivo.
Source: Xu et al. [81].

Micro/nanoencapsulation enhances the sensory properties of functional drinks, including flavor, shelf-life stability, and sustained flavor release. The added encapsulated bioactives further enhance the health benefits of the drinks. An isotonic liquid matrix, such as isotonic beverages, consists of a variety of readily metabolizable carbohydrates and a proper mineral balance and is an ideal medium for delivering functional ingredients in terms of supplementation. Developed an isotonic drink that was fortified with soybean isoflavone microencapsulated with inulin or maltodextrin to investigate the influence of microencapsulation on the stability and antioxidant activity of isoflavones under simulated in vitro gastrointestinal digestion [84].

The isotonic drink did not introduce a matrix effect on microcapsule delivery. Though the employment of inulin as a carrier had yielded capsules with superior surface structure and storage stability, the isoflavone contents in all the samples were greatly diminished by the acidic and basic environments in the gut. However, stability and incorporation of isolated bioactive compounds adversely affect sensory qualities and preservation. Additionally, heat, light, oxygen, acidic or basic pH, and water can put these bioactive compounds at risk, leading to unwanted changes in the products.

Encapsulation with an appropriate coating material can reverse such problems with minimal or no impact on the product's physicochemical and organoleptic properties [84].

Various methods may be used based on the characteristics of the bioactive compound and the target application. Each encapsulation process has its specific benefits and applications, depending on the bioactive compound and the intended functions of the compound. The method will vary based on stability, release pattern, scalability, and target use in the food, pharmaceutical, or cosmetics industries [85].

6.2. Biotransformation

Understanding the metabolism and bioavailability of isoflavone aglycones is crucial in determining their health effects, such as antioxidant activities and possible epigenetic modifications. The complexity of metabolic conversions impacts the physiological activity of isoflavones, emphasizing the importance of accounting for biotransformation when evaluating their benefits. The bioavailability and constitution or arrangement of isoflavones can be reduced by microbial biotransformation. Co-ingestion of lactic acid bacteria (LAB) with isoflavones enhances the production of equol, which increases their activity [86].

O-DMA and 6-hydroxy-O-DMA (6-OH-O-DMA) are produced by lactic acid bacteria (LAB) or their strains through fermentation. Enterococcus hirae AUH-HM195 and Enterococcus faecium INIA P553 were the initial strains reported to yield O-DMA and 6-OH-O-DMA. Recent research identified the production of these compounds by different LAB species in fermented soy drinks, showing that a high percentage of the strains tested were able to produce O-DMA and 6-OH-O-DMA in such systems. Of the tested strains, O-DMA was maximum in the L. mucosae INIA P508 bacterial strain sample at 21.32 ± 1.21 μM, while the Limosilactobacillus reuteri INIA P572 bacterial strain sample produced 24.22 ± 7.65 μM of 6-OH-O-DMA [10].

LAB (Lactic Acid Bacteria) can be a good alternative for producing fermented soy foods, as some strains can break down isoflavones to yield high aglycone concentrations. Daidzein and genistein can be hydrogenated to DHD and DHG, respectively, though some LAB strains show low efficiency for DHD formation, and DHG was undetectable. The conversion efficiency of glycoside isoflavones to aglycones varies widely among species and strains. Fermentation with different LAB strains produced daidzein and genistein in 14–60% ratios; for example, Limosilactobacillus mucosae INIA P508 increased aglycone levels from 8 μM to nearly 460 μM from an initial 780 μM glycoside concentration [10].

The glycosylated isoflavones are less bioactive and bioavailable than the aglycones such as daidzein, genistein, and glycitein. Isoflavones are biotransformed in the gut by flora into bioactive compounds, but only 30–60% of the population possesses the microorganisms required to hydrolyze isoflavone glycosides into aglycones. Current research has explored methods to enhance isoflavone bioavailability. Enzymatic hydrolysis by β-glucosidase is a significant process for hydrolyzing soy isoflavone glycosides. Research has explored β-glucosidases from leguminous plants and microorganisms. Fermentation is also a useful method, enhancing the ratio of the composition of gut bacteria and β-glucosidase activity [86].

Research has shown that fermented foods change gut microbiota composition, produce metabolites, and maintain homeostasis. This enhances the hydrolysis of glycosidic isoflavones to aglycones, increasing their nutritional and functional values. Biotransformation of IFGs during soybean fermentation may provide anticancer candidates. Studies show moderate soy and isoflavone intake reduces breast cancer risk in postmenopausal women. Isoflavones also improve lipid metabolism, reducing LDL, FFA, TG, liver oxidative stress, plasma insulin, blood glucose, insulin resistance, and body weight [86].

6.3. Nanoformulation

First, nanotechnology may alter the various processes that food must undergo, particularly regarding deterioration, by enhancing stability and durability through nanobiotechnology, nanoscale reactions, molecular synthesis, and heat and mass transfer. Secondly, it forms some materials such as nanoparticles, nanoemulsions, nanocomposites, and nanostructures, which may be used for products or packaging. Lastly, nanosensors or nanotracers may be utilized to enhance food security and safety. Nanoencapsulation is the process responsible for encapsulating materials at the nanometer scale. Those materials are trapped within another compound like a capsule; thus, this forms a capsule structure or model. Nanocapsules or nanonospheres are made of a sequence of thin layers, usually spherical, with the ability to encapsulate gases, liquids, or solids inside them, known as the nucleus, active or internal phase. Nanoencapsulation is usually a sophisticated process compared to microcapsules due to the challenge of achieving the proper morphology [83].

Electro-spraying is a process that involves the conversion of a liquid solution into small particles at the nano- and microscale by utilizing an electric field. By utilizing this process, simple nanoparticles, nanocapsules, and polymeric fibers can be prepared. Electrospraying is a new method that utilizes electric fields to produce nanoparticles and nanocapsules of the same size, an important factor for ensuring uniform performance in applications. The coaxial electrospraying method further enhances this capability, with the effective production of nanocapsules while maintaining the bioactivity of sensitive compounds. The process requires very careful control of processing parameters for the attainment of the required properties of nanoparticles. Constant optimization and research would further facilitate the use of electrospraying in food technology and other applications, making improved nanoparticle formulations more convenient [83].

Nanoemulsions are colloidal systems with submicron particles, with sizes ranging from 10 to 1000 nanometers. Carriers exist in the form of solid spherical structures with an amorphous, lipophilic surface, having a negative charge. Nanoemulsion synthesis is performed using different techniques, including the phase inversion method, sonication method, and high-pressure homogenization. Nevertheless, limitations in achieving reduced droplet size and the requirement for specialized equipment and manufacturing processes make the creation of nanoemulsions a costly process [87].

Nanoemulsions are thermodynamically unstable. Particle size, constituent composition, viscosity, density, operational conditions, and the employed technique function to modulate as well as control the globule dimension of nanoemulsions. Emulsion stability increases when the small molecule size is decreased. This improved stability results in enhanced loading capacity, solubility, release profiles, bioavailability, and environmental degradation protection. Liposomes consist of phospholipid molecules, and their structure resembles a closed spherical vesicle. Lipophilic bioactive compounds, including vitamins, polyphenols, and flavonoids, can be entrapped in nanoliposomes through the solubilization process of lipophilic compounds. The bioavailability and activity of bioactive compounds are affected by factors such as temperature, pH, humidity, etc., during processing and storage conditions [88].

Nanomaterials can produce tissue disruption by altering redox potential, which in turn modulates the performance of cells. The effect of nanomaterials on cell health is not fully understood and requires further research to elucidate their activity in cellular environments. Therefore, selecting an appropriate nanocarrier and its corresponding preparation method is essential for controlling the release of the substance. Organic solvents, detergents, or surfactants used in nanoparticle production may leave residues in delivery systems. The successful application of bioactive compounds depends on identifying the safety properties of the nanoparticles. Additionally, there are numerous challenges related to efficacy and bioavailability, as nanomaterials can interact with the nanocarrier, food matrix, and human gastrointestinal system [88].

7. Synergistic Effects of Isoflavone and Probiotic Beverage

The association of isoflavones in probiotic functional drinks shows a combined action, which produces a diverse range of potential health consequences. Both isoflavones and probiotics are shown independently to result in health advantages, while their combination can provide enhanced benefits [89]. The stability of both isoflavones and probiotic cultures within the combined products is a critical factor for their efficacy. Isoflavones and probiotics are noted as promising nutrients for bone wellness, whereby they act by modulating calcium absorption, gut flora, as well as several metabolic processes correlated with osteoblastic activity and bone matrix formation [90, 91].

Specifically, in postmenopausal women suffering from osteopenia, the simultaneous use of bioavailable isoflavones and probiotics was shown to improve bone status as well as estrogen metabolism [9, 68] Intestinal absorption of calcium may also be promoted by them, while bone loss is prevented [68]. Genistein alone or in combination with daidzein is speculated to support bone density [92]. Beyond bone health, their combined or synergistic effects extend to metabolic and cardiovascular well-being. Isoflavones may not only improve lipid profiles but also reduce the risk of cardiovascular disease through both estrogenic and non-estrogenic mechanisms [93].

Studies indicate that soymilk fortified with probiotics offers notable benefits in cardiovascular risk factors in patients with Type 2 Diabetes Mellitus, in indices like blood pressure, total cholesterol (TC), triglycerides (TG), HDL-C, and insulin concentration. Therefore, this highlights its potential advantageous effects on metabolic syndrome [57]. In addition, the interaction between isoflavones and probiotics has a significant impact on gastrointestinal health and overall well-being. In an international multimodal study [94], it was identified that over 40% of individuals worldwide have Functional Gastrointestinal Diseases (FGIDs), which impact quality of life and health care utilization [95]. The gut microbiota plays a central role in isoflavone metabolism, while probiotics enhance their bioavailability by also promoting antioxidant activity in human subjects [96].

Isoflavones are capable of modulating gut microbiota while suppressing disease-causing bacteria proliferation and modulating obesity-linked bacteria strains, an action further augmented by the positive attributes of probiotics [13, 97]. Gut microbiota modulation by such isoflavones can trigger an anti-inflammatory response with delayed progression of disease [98]. Increased isoflavone aglycone bioavailability, which is attained when subjected to fermentation by probiotics, maximizes health effects while minimizing LDL oxidation and inhibiting breast cancer cells [99]. In conclusion, isoflavones and probiotics are a beneficial therapeutic remedy for a variety of health ailments, including menopausal disorders, osteoporosis, and cardiovascular disease. Additionally, they contribute to the improvement of metabolic diseases. These are the synergistic effects of both isoflavones and probiotics in combination with each other.

8. Challenges and Future Directions

We convened to clarify new acute and long-term risks linked with probiotics and to refine recommendations regarding probiotic safety. Probiotic safety involves inherent properties of the probiotic, to the consumer/patient, and to the manufacturing process (safety issue: contaminated probiotic products). Probiotics are applied in both general consumer and clinical aspects. Nevertheless, theoretical and documented adverse effects of probiotic intake do exist. New probiotic strains and products, and broader application of probiotics to at-risk groups, necessitate brief and practical recommendations regarding how to move toward their safe and effective use [100].

The International Scientific Association for Probiotics and Prebiotics organized a meeting to debate and generate evidence-based recommendations for possible acute and long-term hazards, risks to vulnerable populations, the significance of probiotic product quality in relation to meeting the needs of vulnerable populations, and the necessity of adverse event reporting associated with the use of probiotics. The authors note that it is difficult to assess long-term risk due to a lack of data and that there is a requirement for a comparable kind of regulatory scheme as other biologics and continuing research for new safety issues. The article also warns that while microbiome changes have relevance, what their clinical relevance is often remains unclear and would require further studies for the definition of specific high-risk groups that need longer-term follow-up (e.g., pregnant obese women, premature neonates). Finally, the paper recognizes that no intervention carries zero risk and urges all individuals participating in probiotic safety evaluation, scientific, regulatory, and medical communities to take into account the newly emerged problems and assess the risk-benefit ratio, even under current regulatory regimes [100].

8.1. Sensory Challenges

One main obstacle is the sensory characteristics of the drinks. While probiotic fermentation can create the taste of the plant foods and beverages, traditionally, sorghum-based beverages might have unpalatable flavors or beany odors that need to be corrected to improve acceptance by the consumer. Following probiotic fermentation, the unpleasant odor of the okara drink was greatly reduced; e.g., the concentration of the major bean odor compound [2,4-decadienal (E, E)-] was significantly decreased. Upon fermentation, numerous esters were formed, and a natural fruit flavor was produced, positively affecting the okara drink. Above all, the probiotic-fermented okara drink retained viable counts and exhibited good storage stability throughout the storage period. The variety and levels of enzymes and compounds synthesized by various probiotic microbes during metabolism lead to diversified flavors and influence sensory quality. Mixed-strain fermentation has the potential to develop taste, odor, and overall acceptability more than single-strain fermentation in certain instances [12].

8.2. Scalability Issues

Scalability is also a major problem, mainly because of the unstable and short shelf life of live probiotic micro-organisms in drinks [101, 102]. Probiotic survival in non-refrigerated applications is one of the main barriers to mass-market entry. Preservation of probiotic function and gut colonization at commercial production and distribution stages is challenging. Even though there are some potential technologies like encapsulation, safety, scalable, and economical production, issues arise [102]. The probiotics can be destroyed by conditions such as low pH resulting from fermentation, which would also compromise their shelf life over long periods [12].

8.3. Regulatory Compliance

Functional food and probiotic drink regulatory standards are multifaceted and may differ internationally, creating additional obstacles. To meet the product's defined probiotic viability, health claims, and safety requirements, rigorous testing and compliance with established guidelines are necessary [102]. The commercialization of new plant-based products, particularly those involving multifunctional ingredients such as isoflavones and probiotics, requires consensual and clear regulatory guidelines to facilitate their entry into the market and to build consumers' confidence [12].

Prospects for the future of probiotic sorghum drinks include more research optimizing fermentation conditions for higher sensory quality and stable probiotics, exploration of new means of encapsulation for enhanced survivability, and globalization of regulation standards to allow access to more markets [12, 102, 103]. A focus on more sustainable production technologies, such as those based on natural deep eutectic solvents and enzymatic assistance for isoflavone recovery, will also be critically important for the long-term success of these functional beverages [99].

9. Conclusions

The combination of isoflavones together with probiotics in food, such as cereal-based beverage sorghum drink, possesses great potential for human health improvement, especially bone health, through the modulation of calcium absorption and gut microbiota. Fermented cereal-based drinks from African traditional foods, such as those derived from sorghum, have shown promising outcomes in studies as effective delivery vehicles for probiotics. Sorghum beverages can promote the proliferation of probiotic organisms while providing nutritional value; however, further focused research is necessary to fully understand the synergistic action between isoflavones and their combination with probiotics in such specific matrices. Another critical issue in manufacturing plant-source probiotic drinks, including those from sorghum, is maintaining viable probiotic strains during processing and storage. For example, a sorghum-based probiotic beverage demonstrated a 28-day shelf life, but only 14 days with active probiotics, indicating a need for increased stability. Isoflavones are often present in their less absorbable glycoside forms in raw materials; absorption can be significantly enhanced through probiotic fermentation, converting these forms into more active aglycones. This transformation is beneficial to maximize health benefits for consumers. Further research should focus on identifying novel probiotic strains found in traditional African fermented drinks that remain active and effective throughout the digestive system to reach the colon. Additionally, efforts should be directed toward developing controlled fermentation processes with improved starter cultures, ensuring that such drinks are acceptable in taste and contain stable microbes. Scientific advancements in these areas, combined with increased knowledge of health benefits, will be invaluable for the wider acceptance and consumption of probiotic sorghum drinks in regions where grains are a major dietary component [104].

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

Copyright:

References

[1] J. Li, J. Tian, M. Zhou, J. Tian, and C. Liang, "Research progress on the physiological and molecular mechanisms underlying soybean aluminum resistance," *New Crops*, vol. 2, p. 100034, 2025. https://doi.org/10.1016/J.NCROPS.2024.100034

[2] A. F. Bent, "Exploring soybean resistance to soybean cyst nematode," *Annual Review of Phytopathology*, vol. 60, no. 1, pp. 379-409, 2022. https://doi.org/10.1146/ANNUREV-PHYTO-020620-120823/CITE/REFWORKS

[3] P. Qin, T. Wang, and Y. Luo, "A review on plant-based proteins from soybean: Health benefits and soy product development," *Journal of Agriculture and Food Research*, vol. 7, p. 100265, 2022.

[4] S. Bagale, "Nutrient management for soybean crops," *International Journal of Agronomy*, vol. 2021, no. 1, p. 3304634, 2021. https://doi.org/10.1155/2021/3304634

[5] X. Guan *et al.*, "Changes of soybean protein during tofu processing," *Foods*, vol. 10, no. 7, p. 1594, 2021.

[6] S. I. Sohn, S. Pandian, Y. J. Oh, H. J. Kang, W. S. Cho, and Y. S. Cho, "Metabolic engineering of isoflavones: an updated overview," *Frontiers in Plant Science*, vol. 12, p. 670103, 2021. https://doi.org/10.3389/FPLS.2021.670103/XML

[7] S. Liga, C. Paul, and F. Péter, "Flavonoids: Overview of biosynthesis, biological activity, and current extraction techniques," *Plants*, vol. 12, no. 14, p. 2732, 2023.

[8] E. V. Aguiar, F. G. Santos, V. A. V. Queiroz, and V. D. Capriles, "A decade of evidence of sorghum potential in the development of novel food products: Insights from a bibliometric analysis," *Foods*, vol. 12, no. 20, p. 3790, 2023. https://doi.org/10.3390/FOODS12203790

[9] F. Wang, W. Wei, and P. J. Liu, "Effects of probiotic supplementation on bone health in postmenopausal women: A systematic review and meta-analysis," *Frontiers in Endocrinology*, vol. 15, p. 1487998, 2024. https://doi.org/10.3389/fendo.2024.1487998

[10] S. Langa, Á. Peirotén, J. A. Curiel, A. R. de la Bastida, and J. M. Landete, "Isoflavone metabolism by lactic acid bacteria and its application in the development of fermented soy food with beneficial effects on human health," *Foods*, vol. 12, no. 6, p. 1293, 2023. https://doi.org/10.3390/FOODS12061293

[11] W. Li, X. Zhang, S. Wang, X. Gao, and X. Zhang, "Research progress on extraction and detection technologies of flavonoid compounds in foods," *Foods*, vol. 13, no. 4, p. 628, 2024. https://doi.org/10.3390/FOODS13040628

[12] Y. Li *et al.*, "Effects of fermentation with different probiotics on the quality, isoflavone content, and flavor of okara beverages," *Food Science & Nutrition*, vol. 12, no. 4, pp. 2619-2633, 2024.

[13] L. Huang, T. Zheng, H. Hui, and G. Xie, "Soybean isoflavones modulate gut microbiota to benefit the health weight and metabolism," *Frontiers in Cellular and Infection Microbiology*, vol. 12, p. 1004765, 2022.

[14] P. A. Vlaicu, A. E. Untea, I. Varzaru, M. Saracila, and A. G. Oancea, "Designing nutrition for health—Incorporating dietary by-products into poultry feeds to create functional foods with insights into health benefits, risks, bioactive compounds, food component functionality and safety regulations," *Foods*, vol. 12, no. 21, p. 4001, 2023. https://doi.org/10.3390/foods12214001

[15] C. Hu, W.-T. Wong, R. Wu, and W.-F. Lai, "Biochemistry and use of soybean isoflavones in functional food development," *Critical Reviews in Food Science and Nutrition*, vol. 60, no. 12, pp. 2098-2112, 2020.

[16] L. Křížová, K. Dadáková, J. Kašparovská, and T. Kašparovský, "Isoflavones," *Molecules*, vol. 24, no. 6, p. 1076, 2019.

[17] I.-S. Kim, "Current perspectives on the beneficial effects of soybean isoflavones and their metabolites for humans," *Antioxidants*, vol. 10, no. 7, p. 1064, 2021.

[18] R. Negi *et al.*, "Microbial consortia: promising tool as plant bioinoculants for agricultural sustainability," *Current Microbiology*, vol. 81, no. 8, p. 222, 2024. http://doi.org/10.7324/JABB.2024.180889

[19] F. F. Y. Stéphane, B. K. J. Jules, G. E.-S. Batiha, I. Ali, and L. N. Bruno, "Compounds from medicinal plants," *Natural Medicinal Plants*, p. 147, 2022.

[20] Z. Zhang, J. Lv, L. Pan, and Y. Zhang, "Roles and applications of probiotic Lactobacillus strains," *Applied Microbiology and biotechnology*, vol. 102, pp. 8135-8143, 2018. https://doi.org/10.1007/s00253-018-9217-9

[21] C. Q. Zhu *et al.*, "Advances in extraction and purification of citrus flavonoids," *Food Frontiers*, vol. 4, no. 2, pp. 750-781, 2023. https://doi.org/10.1002/fft2.236

[22] E. Quitério, C. Grosso, R. Ferraz, C. Delerue-Matos, and C. Soares, "A critical comparison of the advanced extraction techniques applied to obtain health-promoting compounds from seaweeds," *Marine Drugs*, vol. 20, no. 11, p. 677, 2022. https://doi.org/10.3390/MD20110677

[23] A. S. Dheyab, M. F. Abu Bakar, M. AlOmar, S. F. Sabran, A. F. Muhamad Hanafi, and A. Mohamad, "Deep eutectic solvents (DESs) as green extraction media of beneficial bioactive phytochemicals," *Separations*, vol. 8, no. 10, p. 176, 2021. https://doi.org/10.3390/SEPARATIONS8100176

[24] I. Tariq *et al.*, "A review on extraction technique and immune-boosting properties of Moringa oleifera Lam," *International Journal of Food Properties*, vol. 26, no. 1, pp. 2493-2508, 2023. https://doi.org/10.1080/10942912.2023.2249263

[25] C. Bitwell, S. S. Indra, C. Luke, and M. K. Kakoma, "A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants," *Scientific African*, vol. 19, p. e01585, 2023. https://doi.org/10.1016/J.SCIAF.2023.E01585

[26] S. Cao *et al.*, "Comparative analysis of extraction technologies for plant extracts and absolutes," *Frontiers in Chemistry*, vol. 13, p. 1536590, 2025. https://doi.org/10.3389/FCHEM.2025.1536590/BIBTEX

[27] S. Rayane, Tacila Jericó and L. C. L. d. A. Santana, "Conventional and emerging techniques for extraction of bioactive compounds from fruit waste," *Brazilian Journal of Food Technology*, vol. 25, p. e2021130, 2022.

[28] H. Wang, L. Deng, and G. Huang, "Ultrasound-assisted extraction and value of active substances in Muxu," *Ultrasonics Sonochemistry*, vol. 113, p. 107220, 2025. https://doi.org/10.1016/j.ultsonch.2024.107220

[29] T. C. Pereira, V. P. Souza, A. P. F. Padilha, F. A. Duarte, and E. M. Flores, "Trends and perspectives on the ultrasound-assisted extraction of bioactive compounds using natural deep eutectic solvents," *Current Opinion in Chemical Engineering*, vol. 47, p. 101088, 2025.

[30] N. Mehta *et al.*, "Ultrasound-assisted extraction and the encapsulation of bioactive components for food applications," *Foods*, vol. 11, no. 19, p. 2973, 2022.

[31] T. Xuan *et al.*, "Advances in extraction, purification, and analysis techniques of the main components of kudzu root: A comprehensive review," *Molecules*, vol. 28, no. 18, p. 6577, 2023. https://doi.org/10.3390/molecules28186577

[32] A. Jurinjak Tušek, D. Šamec, and A. Šalić, "Modern techniques for flavonoid extraction—To optimize or not to optimize?," *Applied Sciences*, vol. 12, no. 22, p. 11865, 2022.

[33] M. F. Manzoor *et al.*, "Novel extraction, rapid assessment and bioavailability improvement of quercetin: A review," *Ultrasonics Sonochemistry*, vol. 78, p. 105686, 2021.

[34] P. N. Anyiam *et al.*, "A comparative review on novel-assisted extraction techniques for retrieving protein from some potential plant sources," *NFS Journal*, p. 100239, 2025. https://doi.org/10.1016/J.NFS.2025.100239

[35] A. Łubek-Nguyen, W. Ziemichód, and M. Olech, "Application of enzyme-assisted extraction for the recovery of natural bioactive compounds for nutraceutical and pharmaceutical applications," *Applied Sciences*, vol. 12, no. 7, p. 3232, 2022.

[36] M. Usman, M. Nakagawa, and S. Cheng, "Emerging trends in green extraction techniques for bioactive natural products," *Processes*, vol. 11, no. 12, p. 3444, 2023. https://doi.org/10.3390/pr11123444

[37] M. Yıldırım, M. Erşatır, S. Poyraz, M. Amangeldinova, N. O. Kudrina, and N. V. Terletskaya, "Green extraction of plant materials using supercritical CO2: insights into methods, analysis, and bioactivity," *Plants*, vol. 13, no. 16, p. 2295, 2024. https://doi.org/10.3390/plants13162295

[38] F. Herzyk, D. Piłakowska-Pietras, and M. Korzeniowska, "Supercritical extraction techniques for obtaining biologically active substances from a variety of plant byproducts," *Foods*, vol. 13, no. 11, p. 1713, 2024.

[39] H. Fraguela-Meissimilly *et al.*, "New trends in supercritical fluid technology and pressurized liquids for the extraction and recovery of bioactive compounds from agro-industrial and marine food waste," *Molecules*, vol. 28, no. 11, p. 4421, 2023. https://doi.org/10.3390/MOLECULES28114421

[40] J. A. Almohasin, J. Balag, V. G. Miral, R. V. Moreno, L. J. Tongco, and E. C. R. Lopez, "Green solvents for liquid–liquid extraction: Recent advances and future trends," *Engineering Proceedings*, vol. 56, no. 1, p. 174, 2023. https://doi.org/10.3390/ASEC2023-16278

[41] A. da Silva Vale *et al.*, "Exploring microbial diversity of non-dairy fermented beverages with a focus on functional probiotic microorganisms," *Fermentation*, vol. 9, no. 6, p. 496, 2023. https://doi.org/10.3390/fermentation9060496

[42] M. Ziarno and P. Cichońska, "Lactic acid bacteria-fermentable cereal-and pseudocereal-based beverages," *Microorganisms*, vol. 9, no. 12, p. 2532, 2021. https://doi.org/10.3390/microorganisms9122532

[43] A. Stefoska-Needham, "Sorghum and health: An overview of potential protective health effects," *Journal of Food Science*, vol. 89, no. S1, pp. A30-A41, 2024. https://doi.org/10.1111/1750-3841.16978

[44] A. K. Rashwan, H. A. Yones, N. Karim, E. M. Taha, and W. Chen, "Potential processing technologies for developing sorghum-based food products: An update and comprehensive review," *Trends in Food Science & Technology*, vol. 110, pp. 168-182, 2021. https://doi.org/10.1016/J.TIFS.2021.01.087

[45] J. K. Ndukwe *et al.*, "Diet diversification and priming with Kunu: An indigenous probiotic cereal-based non-alcoholic beverage in Nigeria," *Beverages*, vol. 9, no. 1, p. 14, 2023.

[46] S. Bheemaiah Balyatanda, N. N. Gowda, J. Subbiah, S. Chakraborty, P. V. Prasad, and K. Siliveru, "Physiochemical, bio, thermal, and non-thermal processing of major and minor millets: A comprehensive review on antinutritional and antioxidant properties," *Foods*, vol. 13, no. 22, p. 3684, 2024. https://doi.org/10.3390/FOODS13223684

[47] Z. Li, X. Zhao, X. Zhang, and H. Liu, "Bioactive compounds and biological activities of sorghum grains," *Foods*, vol. 10, no. 11, p. 2868, 2021.

[48] L. M. Paucar-Menacho *et al.*, "Performance of thermoplastic extrusion, germination, fermentation, and hydrolysis techniques on phenolic compounds in cereals and pseudocereals," *Foods*, vol. 11, no. 13, p. 1957, 2022.

[49] M. Rodríguez-España, C. Y. Figueroa-Hernández, J. de Dios Figueroa-Cárdenas, P. Rayas-Duarte, and Z. J. Hernández-Estrada, "Effects of germination and lactic acid fermentation on nutritional and rheological properties of sorghum: A graphical review," *Current Research in Food Science*, vol. 5, pp. 807-812, 2022. https://doi.org/10.1016/J.CRFS.2022.04.014

[50] B. Li *et al.*, "Flavonoids mitigation of typical food thermal processing contaminants: Potential mechanisms and analytical strategies," *Food Chemistry*, vol. 416, p. 135793, 2023. https://doi.org/10.1016/J.FOODCHEM.2023.135793

[51] J. Xu, W. Wang, and Y. Zhao, "Phenolic compounds in whole grain sorghum and their health benefits," *Foods*, vol. 10, no. 8, p. 1921, 2021. https://doi.org/10.3390/foods10081921

[52] J. Joshi, S. S. Kumar, R. K. Rout, and P. S. Rao, "Millet processing: prospects for climate-smart agriculture and transition from food security to nutritional security," *Journal of Future Foods*, vol. 5, no. 5, pp. 470-479, 2025.

[53] F. Mahfoud *et al.*, "Evolution of dried food texturization: A critical review of technologies and their impact on organoleptic and nutritional properties," *Processes*, vol. 13, no. 3, p. 799, 2025.

[54] S. H. Saeed Omer, J. Hong, X. Zheng, and R. Khashaba, "Sorghum flour and sorghum flour enriched bread: characterizations, challenges, and potential improvements," *Foods*, vol. 12, no. 23, p. 4221, 2023. https://doi.org/10.3390/FOODS12234221

[55] J. Zhang, P. Li, X. Yang, and L. Wang, "The antinutritional factors and technological processing of sorghum and its application in pig production," *Animals*, vol. 15, no. 3, p. 328, 2025. https://doi.org/10.3390/ani15030328

[56] D. Santiago-Ramos, J. de Dios Figueroa-Cárdenas, R. M. Mariscal-Moreno, A. Escalante-Aburto, N. Ponce-García, and J. J. Véles-Medina, "Physical and chemical changes undergone by pericarp and endosperm during corn nixtamalization-A review," *Journal of Cereal Science*, vol. 81, pp. 108-117, 2018. https://doi.org/10.1016/J.JCS.2018.04.003

[57] S. Hassan, S. Forsido, Y. Tola, A. Bikila, and Z. Ahmed, "Effect of nixtamalization on the nutritional, anti-nutritional, functional, physicochemical and mineral properties of maize (Zea mays) tortillas," *Journal of Food Chemistry and Nanotechnology*, vol. 9, no. 3, pp. 132-140, 2023.

[58] S. N. Nguyen, P. Drawbridge, and T. Beta, "Distribution of cereal phytochemicals and micronutrients in whole grains: A review of nutraceutical, industrial, and agricultural implications," *Cereal Chemistry*, vol. 101, no. 5, pp. 903-925, 2024.

[59] A. Khoddami, V. Messina, K. Vadabalija Venkata, A. Farahnaky, C. L. Blanchard, and T. H. Roberts, "Sorghum in foods: Functionality and potential in innovative products," *Critical Reviews in Food Science and Nutrition*, vol. 63, no. 9, pp. 1170-1186, 2023.

[60] E.-S. M. Abdel-Aal, "Insights into grain milling and fractionation practices for improved food sustainability with emphasis on wheat and peas," *Foods*, vol. 13, no. 10, p. 1532, 2024. https://doi.org/10.3390/FOODS13101532

[61] D. A. Kitessa, "Review on effect of fermentation on physicochemical properties, anti-nutritional factors and sensory properties of cereal-based fermented foods and beverages," *Annals of Microbiology*, vol. 74, no. 1, p. 32, 2024.

[62] L. Cuamatzin-García *et al.*, "Traditional fermented foods and beverages from around the world and their health benefits," *Microorganisms*, vol. 10, no. 6, p. 1151, 2022. https://doi.org/10.3390/MICROORGANISMS10061151

[63] S. M. Rapoo and M.-L. Thaoge-Zwane, "Amylolytic capability and performance of probiotic strains in a controlled sorghum fermentation system," *Fermentation*, vol. 10, no. 6, p. 308, 2024. https://doi.org/10.3390/FERMENTATION10060308/S1

[64] S. Bouakkaz, H. Zerizer, K. Rachedi, A. Accettulli, A. Racioppo, and A. Bevilacqua, "African cereal-based fermented foods: Microbiota, functional microorganisms, starter cultures and nutritional properties," *Food Bioscience*, vol. 62, p. 105212, 2024. https://doi.org/10.1016/J.FBIO.2024.105212

[65] H. Hawaz, B. Bottari, F. Scazzina, and E. Carini, "Eastern African traditional fermented foods and beverages: Advancements, challenges, and perspectives on food technology, nutrition, and safety," *Comprehensive Reviews in Food Science and Food Safety*, vol. 24, no. 2, p. e70137, 2025.

[66] L. Day, R. B. Seymour, K. F. Pitts, I. Konczak, and L. Lundin, "Incorporation of functional ingredients into foods," *Trends in Food Science & Technology*, vol. 20, no. 9, pp. 388-395, 2009. https://doi.org/10.1016/J.TIFS.2008.05.002

[67] Q. Raza, M. T. Azhar, I. A. Rana, M. Q. Ahmad, and R. M. Atif, "Biofortification of crops to achieve food and nutritional security," *Biofortification of Grain and Vegetable Crops*, pp. 1-17, 2024. https://doi.org/10.1016/B978-0-323-91735-3.00001-7

[68] I. A. Harahap, M. Kuligowski, M. Schmidt, P. A. Kołodziejski, and J. Suliburska, "Effects of isoflavone and probiotic intake on calcium transport and bone metabolism biomarkers in female rats," *Food Science & Nutrition*, vol. 11, no. 10, pp. 6324-6335, 2023.

[69] N. Labeling, "Overview of food fortification in the United States and Canada," 2003. https://www.ncbi.nlm.nih.gov/books/NBK208880/

[70] L. A. Berner, D. R. Keast, R. L. Bailey, and J. T. Dwyer, "Fortified foods are major contributors to nutrient intakes in diets of US children and adolescents," *Journal of the Academy of Nutrition and Dietetics*, vol. 114, no. 7, pp. 1009-1022. e8, 2014. https://doi.org/10.1016/J.JAND.2013.10.012

[71] T. Beal, S. Manohar, L. Miachon, and J. Fanzo, "Nutrient-dense foods and diverse diets are important for ensuring adequate nutrition across the life course," *Proceedings of the National Academy of Sciences*, vol. 121, no. 50, p. e2319007121, 2024. https://doi.org/10.1073/PNAS.2319007121

[72] S. Schaarschmidt and C. Fauhl-Hassek, "Mycotoxins during the processes of nixtamalization and tortilla production," *Toxins*, vol. 11, no. 4, p. 227, 2019. https://doi.org/10.3390/TOXINS11040227

[73] U.S. Food and Drug Administration, "Title of guidance document. U.S. Department of Health and Human Services," 2024. https://www.fda.gov/

[74] M. P. Caporgno and A. Mathys, "Trends in microalgae incorporation into innovative food products with potential health benefits," *Frontiers in Nutrition*, vol. 5, p. 386576, 2018. https://doi.org/10.3389/FNUT.2018.00058/BIBTEX

[75] C. w. Yu and Q. r. Hu, "Incorporation of dietary phenolic compounds into dairy products: Interactions and beneficial effects," *International Journal of Dairy Technology*, vol. 78, no. 1, p. e13161, 2025. https://doi.org/10.1111/1471-0307.13161

[76] H. Demmelmair *et al.*, "The effect of Atlantic salmon consumption on the cognitive performance of preschool children–a randomized controlled trial," *Clinical Nutrition*, vol. 38, no. 6, pp. 2558-2568, 2019. https://doi.org/10.1016/j.clnu.2018.11.031

[77] D. Nelson *et al.*, "First results from the IllustrisTNG simulations: The galaxy colour bimodality," *Monthly Notices of the Royal Astronomical Society*, vol. 475, no. 1, pp. 624-647, 2018.

[78] D. R. Jacobs Jr, M. D. Gross, and L. C. Tapsell, "Food synergy: An operational concept for understanding nutrition," *The American Journal of Clinical Nutrition*, vol. 89, no. 5, pp. 1543S-1548S, 2009.

[79] A. Drewnowski *et al.*, "Perspective: why whole grains should be incorporated into nutrient-profile models to better capture nutrient density," *Advances in Nutrition*, vol. 12, no. 3, pp. 600-608, 2021. https://doi.org/10.1093/ADVANCES/NMAA172

[80] S. M. Jafari, *Nanoencapsulation of food bioactive ingredients: Principles and applications*. Cambridge, MA, USA: Academic Press, 2017.

[81] Y. Xu *et al.*, "The application of encapsulation technology in the food Industry: Classifications, recent Advances, and perspectives," *Food Chemistry: X*, vol. 21, p. 101240, 2024. https://doi.org/10.1016/j.fochx.2024.101240

[82] G. Ozkan *et al.*, "Encapsulated phenolic compounds: Clinical efficacy of a novel delivery method," *Phytochemistry Reviews*, vol. 23, no. 3, pp. 781-819, 2024.

[83] K. H. Ozuna-Valencia *et al.*, "The application of organic and inorganic nanoparticles incorporated in edible coatings and their effect on the physicochemical and microbiological properties of seafood," *Processes*, vol. 12, no. 9, p. 1889, 2024.

[84] H. J. Qazi, A. Ye, A. Acevedo-Fani, and H. Singh, "Delivery of encapsulated bioactive compounds within food matrices to the digestive tract: recent trends and future perspectives," *Critical Reviews in Food Science and Nutrition*, vol. 65, no. 15, pp. 2921-2942, 2025.

[85] T. S. d. C. Oliveira *et al.*, "Bioactive compounds and the performance of proteins as wall materials for their encapsulation," *Micro*, vol. 5, no. 3, p. 36, 2025.

[86] S. Nurmilah, A. Frediansyah, Y. Cahyana, and G. L. Utama, "Biotransformation and health potential of isoflavones by microorganisms in Indonesian traditional fermented soy products: A review," *Journal of Agriculture and Food Research*, vol. 18, p. 101365, 2024.

[87] S. Singh *et al.*, "Unveiling the pharmacological and nanotechnological facets of daidzein: Present state-of-the-art and future perspectives," *Molecules*, vol. 28, no. 4, p. 1765, 2023. https://doi.org/10.3390/MOLECULES28041765

[88] M. G. Kamble, A. Singh, S. V. Singh, M. G. Kamble, N. A. Sagar, and N. Rani, "Nanotechnology for encapsulation of bioactive components: A review," *Discover Food*, vol. 5, no. 1, pp. 1-18, 2025.

[89] A. E. Ribeiro, N. E. S. Monteiro, A. V. G. de Moraes, L. H. Costa-Paiva, and A. O. Pedro, "Can the use of probiotics in association with isoflavone improve the symptoms of genitourinary syndrome of menopause? Results from a randomized controlled trial," *Menopause*, vol. 26, no. 6, pp. 643-652, 2019. https://doi.org/10.1097/GME.0000000000001279
[90] I. A. Harahap and J. Suliburska, "Probiotics and isoflavones as a promising therapeutic for calcium status and bone health: A narrative review," *Foods*, vol. 10, no. 11, p. 2685, 2021.
[91] H. H. Bashir, M. A. Hasnain, A. Abbas, J.-H. Lee, and G.-S. Moon, "The impact of fermented dairy products and probiotics on bone health improvement," *Food Science of Animal Resources*, vol. 45, no. 2, p. 449, 2025. https://doi.org/10.5851/KOSFA.2025.E7
[92] S. Gómez-Zorita, M. González-Arceo, A. Fernández-Quintela, I. Eseberri, J. Trepiana, and M. P. Portillo, "Scientific evidence supporting the beneficial effects of isoflavones on human health," *Nutrients*, vol. 12, no. 12, p. 3853, 2020. https://doi.org/10.3390/NU12123853
[93] D. Cardoso Umbelino Cavallini *et al.*, "Probiotic soy product supplemented with isoflavones improves the lipid profile of moderately hypercholesterolemic men: A randomized controlled trial," *Nutrients*, vol. 8, no. 1, p. 52, 2016. https://doi.org/10.3390/NU8010052
[94] M. Afzaal *et al.*, "Human gut microbiota in health and disease: Unveiling the relationship," *Frontiers in Microbiology*, vol. 13, p. 999001, 2022. https://doi.org/10.3389/FMICB.2022.999001/XML
[95] A. D. Sperber *et al.*, "Worldwide prevalence and burden of functional gastrointestinal disorders, results of Rome Foundation Global Study," *Gastroenterology*, vol. 160, no. 1, pp. 99-114. e3, 2021. https://doi.org/10.1053/j.gastro.2020.04.014
[96] M. Amidžić *et al.*, "Antioxidant effects of soy isoflavones, probiotics and their combination on carbon tetrachloride-induced oxidative stress in rats," *International Journal of Pharmacology*, vol. 20, no. 4, pp. 561-572, 2024. https://doi.org/10.3923/ijp.2024.561.572
[97] P. Chen *et al.*, "The bioavailability of soy isoflavones in vitro and their effects on gut microbiota in the simulator of the human intestinal microbial ecosystem," *Food Research International*, vol. 152, p. 110868, 2022. https://doi.org/10.1016/J.FOODRES.2021.110868
[98] S. Ghimire *et al.*, "Dietary isoflavones alter gut microbiota and lipopolysaccharide biosynthesis to reduce inflammation," *Gut Microbes*, vol. 14, no. 1, p. 2127446, 2022.
[99] F. Makkliang *et al.*, "Biocompatible natural deep eutectic solvent-based extraction and cellulolytic enzyme-mediated transformation of Pueraria mirifica isoflavones: a sustainable approach for increasing health-bioactive constituents," *Bioresources and Bioprocessing*, vol. 8, no. 1, p. 76, 2021.
[100] D. Merenstein *et al.*, "Emerging issues in probiotic safety: 2023 perspectives," *Gut Microbes*, vol. 15, no. 1, p. 2185034, 2023. https://doi.org/10.1080/19490976.2023.2185034;PAGE:STRING:ARTICLE/CHAPTER
[101] A. Abbasi, V. Sarabi-Aghdam, M. Fathi, and S. Abbaszadeh, "Non-dairy fermented probiotic beverages: A critical review on the production techniques, health benefits, safety considerations and market trends," *Food Reviews International*, pp. 1-56, 2025. https://doi.org/10.1080/87559129.2025.2550596
[102] S. Bajkacz and J. Adamek, "Evaluation of new natural deep eutectic solvents for the extraction of isoflavones from soy products," *Talanta*, vol. 168, pp. 329-335, 2017. https://doi.org/10.1016/J.TALANTA.2017.02.065
[103] A. Rashidinejad, "The road ahead for functional foods: Promising opportunities amidst industry challenges," *Future Postharvest and Food*, vol. 1, no. 2, pp. 266-273, 2024. https://doi.org/10.1002/FPF2.12022
[104] M. Setta, A. Matemu, and E. R. Mbega, "Potential of probiotics from fermented cereal-based beverages in improving health of poor people in Africa," *Journal of Food Science and Technology*, vol. 57, no. 11, pp. 3935-3946, 2020.